DISSERTATION ON

IMMUNOHISTOCHEMICAL EVALUATION OF COLORECTAL MALIGNANCIES

A STUDY OF 100 CASES

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CERTIFICATE

This is to certify that this dissertation titled **"IMMUNOHISTOCHEMICAL EVALUATION OF COLORECTAL MALIGNANCIES – A STUDY OF 100 CASES"** is the original and bonafide work done by **Dr. V. Sindu** under the guidance of Dr. R. Padmavathi, M.D., Professor, Department of Pathology at the Government Stanley Medical College & Hospital, Chennai – 600 001, during the tenure of her course in M.D. Pathology from May-2009 to April-2012 held under the regulation of the Tamilnadu Dr. M.G.R. Medical University, Guindy, Chennai - 600032.

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CONTENTS

ABBREVIATIONS

HPE	-	Histopathological examination
CRC	-	Colorectal carcinoma
IHC	-	Immunohistochemistry
HRP	-	Horse raddish peroxidase
WHO	-	World Health Organisation
LOH	-	Loss of heterozygosity
BPR	-	Bleeding per rectum
ALT BH	-	Altered bowel habits
LOW	-	Loss of weight
CT	-	Computerised tomography
PET	-	Positron emission tomography
EDTA	-	Ethylene diamine tetra acetic acid

INTRODUCTION

INTRODUCTION

Colorectal cancer is the third most common cancer worldwide, with an estimated 130,000 new patients diagnosed each year. It is the second leading cause of death because of cancer (approximately 55,000 people annually)[1]. In India the incidence of colorectal cancer is 3.9 per one lakh population and the mortality rate is 2.8 per one lakh population[2]. About 60% of all patients diagnosed with colorectal carcinoma will present with locally advanced disease[3]. Appropriate therapeutic decision making for these individuals depends primarily on the depth of penetration of the primary tumor and metastatic disease in the regional lymph nodes[3].

NORMAL LARGE BOWEL

The large bowel comprises the terminal 1 to 1.5 meters of the gastrointestinal tract and is divided into caecum, ascending colon, transverse colon, descending colon, sigmoid colon & rectum.

HISTOLOGY

The large bowel wall is composed of four layers

1. Mucosa

   Epithelium

   Lamina propria

   Muscularis mucosa

2. Sub mucosa

3. Muscularis propria
4. Serosa

The mucosa is lined by columnar epithelium into which the crypts open. The surface epithelium is composed of absorptive cells and goblet cells. In addition the surface epithelium contains immature and undifferentiated cells, endocrine cells and paneth cells. The epithelium overlying the lymphoid follicles of lamina propria contains follicle associated epithelial cells or 'M' cells (M for microfold or membrane cells).

The absorptive cells have basally located nuclei, mucin negative acidophilic cytoplasm and luminally directed apical striated borders. They absorb excess water and electrolytes from the intestinal contents. The goblet cells synthesize, store and secrete mucin granules. The paneth cells are seen only in caecum and proximal right colon and they secrete lysozyme and epidermal growth factor. Lymphocytes and occasional eosinophils may be present between the surface epithelial cells. The crypts are tubular and are arranged parallel to each other.

The lamina propria contains few lymphocytes (both T and B cells), plasma cells, histiocytes and mast cells scattered in a network of collagen fibers, smooth muscle bundles, vessels and nerves. Lymphoglandular complexes are normal structures formed by deep crypt epithelium surrounded by lymphoid follicles which extend from mucosa to submucosa. Pericryptal fibroblast sheath is a collection of fibroblasts and myofibroblasts located around the crypts in superficial lamina propria.

The muscularis mucosa is composed of thin layer of smooth muscles. The submucosa is composed of loose connective tissue similar to that of lamina propria. It

also contains submucosal plexus of Meissner and often contains fat cells. The muscularis propria is composed of inner circular and outer longitudinal layers of smooth muscles with the myenteric plexus of Auerbach between them. The outer layer is collected to form three thick bands known as taenia coli. The taenia is shorter in length than the other layers of the colonic wall. This results in the production of sacculations or haustrations on the wall of the colon.

The serosa is composed of a single layer of flattened to cuboidal mesothelial cells and the subjacent fibroelastic tissue. It forms small pouches filled with fat known as appendices epiploicae. The serosa is missing over the posterior aspect of ascending and descending colon.

THE VERMIFORM APPENDIX

The appendix is the narrowest part of the gut. The crypts are poorly formed. The longitudinal muscle coat is complete and thick all around. Taenia coli are absent. The submucosa contains abundant lymphoid tissue that may completely fill it. The lymphoid tissue is not present at birth, it gradually increases and it is best seen in children above 10 years of age. Subsequently there is progressive reduction in quantity of lymphoid tissue.

THE RECTUM

The rectum has a continuous coat of longitudinal muscle and there is no taenia coli. The peritoneum covers the front and sides of the upper one-third and only the front of the middle third. The rest of the rectum is devoid of a serous covering.

WHO CLASSIFICATION OF PRIMARY TUMOURS OF COLON AND RECTUM [4]

Tumours of the colorectal region are classified pathologically into different types which are based on the microscopic features.

Epithelial tumours	Non-epithelial tumours
I. Adenoma a. Tubular b. Villous c. Tubulovillous d. Serrated II. Intraepithelial neoplasia a. Low grade b. High grade III. Carcinoma a. Adenocarcinoma b. Mucinous carcinoma	I. Lipoma II. Leiomyoma III. Gastrointestinal stromal tumour IV. Leiomyosarcoma V. Angiosarcoma VI. Kaposi sarcoma VII. Malignant melanoma VIII. Malignant lymphoma IX. Marginal zone lymphoma X. Mantle cell lymphoma XI. Diffuse large B cell lymphoma XII. Burkitt lymphoma

c. Signet ring cell carcinoma
d. Small cell carcinoma
e. Squamous cell carcinoma
f. Adenosquamous carcinoma
g. Medullary carcinoma
h. Undifferentiated carcinoma

IV. Carcinoid
a. Enterochromaffin cell
b. L cell
c. Others

V. Mixed carcinoid - adenocarcinoma

ADENOCARCINOMA

Adenocarcinoma is the most common tumour type. Most are well to moderately differentiated and lack specific histological features, although colorectal tumours tend to show cribriform patterns with central necrosis, a feature that is useful if a metastatic tumour is encountered with an occult colorectal primary. Dysplasia in adjacent mucosa may be seen, but frequently the invasive tumour obliterates any pre-existing polyp from which it may have arisen[5].

MUCINOUS ADENOCARCINOMA

This is one of the subtypes of adenocarcinoma that secretes extracellular mucin. At least 50% of the tumour must be mucinous in order to make this diagnosis. Mucinous adenocarcinomas are associated with microsatellite instability. Whether mucinous tumours have a better prognosis is uncertain. Mucinous change may also be seen in conventional adenocarcinomas treated with neoadjuvant chemoradiotherapy[6].

SIGNET RING CELL CARCINOMA

Signet ring cell carcinoma is composed of at least 50% cells with intracytoplasmic mucin and eccentrically placed nuclei, resembling gastric signet ring cell tumours. The tumour grows in a diffuse fashion[7].

SMALL CELL CARCINOMA

Small cell carcinoma may have areas of glandular or squamous differentiation. The prognosis is extremely poor[8].

SQUAMOUS AND ADENOSQUAMOUS CARCINOMAS

These are extremely rare tumours. They have been associated with ulcerative colitis, schistosomiasis and pelvic irradiation. The survival rates are similar to adenocarcinomas. The following criteria is essential for making the diagnosis of squamous or adenosquamous carcinomas

- There must be no other sites of squamous cell carcinoma in the body
- There must be no involvement of cloacogenic or squamous lined mucosa[9].

MEDULLARY CARCINOMA

This is an important subtype of colorectal cancer, added to the WHO classification in 2000. It has a characteristic phenotype with sheets of cells and numerous tumour infiltrating lymphocytes on microscopy. This phenotype is associated with the Lynch cancer family syndrome (Hereditary Non-Polyposis Colorectal Cancer). Patients with this syndrome may also have ovarian, endometrial, skin and other gastrointestinal tumours. The colorectal tumours show a loss of mismatch repair proteins such as MSH (MutS, Escherichia coli, Homolog in 60% of cases) or MLH (MutL, Escherichia coli, Homolog in 30% of cases), which can be demonstrated with immunohistochemistry[10].

UNDIFFERENTIATED CARCINOMA

They represent less than 1% of colorectal cancers. They are malignant epithelial tumours that have no glandular or other features to indicate definite differentiation. The absence of intracytoplasmic mucin helps to differentiate these tumours from poorly differentiated adenocarcinomas[11].

CARCINOIDS AND NEUROENDOCRINE CARCINOMA

They are rare in the colon but focal neuroendocrine differentiation can occur in conventional adenocarcinomas. These are commonly seen in caecum and rectum[12].

MIXED CARCINOID - ADENOCARCINOMA

They originate from the endodermally derived multipotential cells located at the base of the crypts which during the neoplastic transformation undergo differentiation along several different pathways[13].

MESENCHYMAL TUMOURS

Mesenchymal tumors in the colorectum are rare. Histologically they resemble their counterparts in soft tissues. Leiomyoma is the commonest mesenchymal tumour[14].

LYMPHOMA

Lymphomas are less frequent in large bowel. They are nearly always of non-Hodgkin's type. Most of them belong to MALT-type (Mucosa Associated Lymphoid Tissue) category[15].

METASTATIC TUMOURS

Metastases form disc like areas with central ulceration. They originate from malignant melanoma, lung tumours, renal cell carcinoma or mesothelioma.[16]

Colorectal adenocarcinomas affect males slightly more than females and the mean age of incidence is 62 years. Both environmental and genetic factors are involved in the cause and pathogenesis.

There are a number of genetic mutations involved in colorectal carcinogenesis. These can be assessed by immunohistochemistry. Mutations in p53 have been found to

occur in 70% to 80% of patients with colon cancer[1]. This p53 mutation was proposed as a late event in the transition from adenoma to carcinoma.

Preclinical investigations have demonstrated that mutant p53 renders malignant cells less sensitive to most chemotherapeutic agents, with the exception of the taxanes, which seem to be indifferent to p53 status. The purpose of this study is to analyse the age, sex and site distribution in colorectal adenocarcinomas in our institution and to investigate the level of expression of P53 in colorectal adenocarcinomas and correlate this with the histological grade and stage.

AIMS
AND
OBJECTIVES

AIMS AND OBJECTIVES

1. To analyse age and sex distribution of colorectal adenocarcinomas
2. To analyse site of distribution of colorectal adenocarcinomas
3. To assess the level of expression of p53 in colorectal adenocarcinomas.
4. To correlate the level of expression of p53 with the grade and stage of colorectal adenocarcinomas

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Colorectal carcinoma is a major cause of mortality and morbidity worldwide. Colorectal adenocarcinoma accounts for over 90% of the malignant tumors of the large bowel [17]. Incidence rates of colorectal cancer are increasing in many countries. Unfortunately, despite improvements in medical and surgical provision, there has been comparatively little change in mortality from colorectal cancer during the past 40 years and the overall five year survival is only around 40% [18].

The cause and pathogenesis of colorectal adenocarcinoma are related to both environmental and genetic factors, the former are largely dietary. The factors most closely associated with increased colorectal cancer rates include low intake of unabsorbable vegetable fibers and high intake of refined carbohydrates and fat. The reduced fiber content leads to decreased stool bulk and altered composition of intestinal microbial flora. This change may increase the synthesis of toxic oxidative by-products of bacterial metabolism which act as carcinogens[1].

Deficiency of vitamin A C, E which act as anti-oxidants & high fat intake which enhances the hepatic synthesis of cholesterol and bile acids that are converted into carcinogens by intestinal bacteria are also implicated as risk factors for colorectal carcinomas[1,19]. The genetic factors include mutations of APC (Adenomatous Polyposis Coli), K-RAS, p53, and mismatch repair genes[1,20].

STAGING OF COLORECTAL ADENOCARCINOMAS

Staging of colorectal cancer is an estimate of the amount of penetration of the tumour. It is performed for diagnostic and research purposes and to determine the best method of treatment. The systems for the staging of colorectal cancers depend on the extent of local invasion, the degree of lymph node involvement and whether there is distant metastasis.

Definitive staging can only be done after surgery has been performed and pathology reports are reviewed. An exception to this principle would be after a colonoscopic polypectomy of a malignant pedunculated polyp with minimal invasion. Preoperative staging of rectal cancers may be done with endoscopic ultrasound. Additional investigations which are useful in detecting metastases include abdominal ultrasound, CT, PET scanning and other imaging studies.

The most common and most widely used staging system is the TNM (for tumors/nodes/metastases) system, from the American Joint Committee on Cancer (AJCC). The TNM system assigns a number based on three categories. "T" denotes the degree of invasion of the intestinal wall, "N" the number of lymph node involvement, and "M" the presence or absence of metastases. The broader stage of a cancer is usually quoted as number I, II, III, IV derived from the TNM value grouped by prognosis, a higher number indicates a more advanced cancer and likely a worse outcome. Details of this system are in the table below:

AJCC	TNM STAGE	TNM stage criteria for colorectal cancer[21]
Stage 0	Tis N0 M0	Tis: Tumor confined to mucosa; cancer-in-situ
Stage I	T1 N0 M0	T1: Tumor invades submucosa
	T2 N0 M0	T2: Tumor invades Muscularis propria
Stage II-A	T3 N0 M0	T3: Tumor invades subserosa or beyond (without other organs involved)
Stage II-B	T4 N0 M0	T4: Tumor invades adjacent organs or perforates the visceral peritoneum
Stage III-A	T1-2 N1 M0	N1: Metastasis to 1 to 3 regional lymph nodes.
Stage III-B	T3-4 N1 M0	N1: Metastasis to 1 to 3 regional lymph nodes.
Stage III-C	any T, N2 M0	N2: Metastasis to 4 or more regional lymph nodes.
Stage IV	any T, any N, M1	M1: Distant metastases present.

DUKE'S SYSTEM [22]

Duke's classification is an older and less complicated staging system that predates the TNM system. It identifies the stages as:

- A - Tumour confined to the intestinal wall
- B - Tumour invading through the intestinal wall
- C - With lymph node(s) involvement (this is further subdivided into C1 where only the regional nodes are involved and C2 where the nodes at the point of mesenteric blood vessel ligature are involved)
- D - With distant metastasis

A few cancer centers still use this staging system.

ASTLER-COLLER STAGING SYSTEM[22]

- A: Tumor limited to the mucosa

- B1: Tumor involving the muscularis externa but not penetrating it
- B2: Tumor penetrating through the muscularis externa
- C1: Tumor confined to the bowel wall with regional lymph node metastases
- C2: Tumor penetrating through the wall with regional lymph node metastases
- D: Distant metastases.

Additional Staging:

- Venous invasion (V)
  - V0 : No venous invasion
  - V1 : Microscopic venous invasion
  - V2 : Macroscopic venous invasion
- Lymphatic invasion (L)
  - L0 : No lymphatic vessel invasion
  - L1 : Lymphatic vessel invasion.

J. Walker et al[23], calculated 5 year survival rates in colorectal carcinoma patients who underwent surgery followed by chemotherapy or radiotherapy and correlated with the stage of the disease. He concluded in his study that histopathological staging is at present the most accurate prognostic factor for survival and recurrence.

HISTOPATHOLOGICAL GRADING OF COLORECTAL ADENOCARCINOMAS [24]

Histological grade describes how closely the tumor cells resemble normal cells. In general, the grading of colorectal carcinoma is based on architectural features, as well as cytological features (e.g., pleomorphism and hyperchromatism). However, the degree of gland formation is widely regarded as the most important feature in grading. There are three grades in colorectal adenocarcinomas:

- Grade 1: Also called well differentiated. The tumour is composed mainly of simple tubules or glands in which nuclear polarity is easily discerned and the nuclei are of uniform size.
- Grade 2: Also called moderately differentiated. The tumour is composed of tubules that may be simple, complex or slightly irregular in which nuclear polarity is barely discernible or is lost.
- Grade 3: Also called poorly differentiated. The tumour is predominantly composed of solid pattern and there is loss of nuclear polarity.

P53[25]

P53 is a tumor suppressor gene that is located on chromosome 17p13. It is the single most common target for genetic alteration in human tumors. The product of p53 gene is a phosphoprotein that is activated by DNA damage and involved in the process to determine whether the cells should stop replication or die by apoptosis. In total p53 targets around 150 genes to prevent proliferation of damaged cells. As such p53 has been described as “the guardian of the genome”, the "guardian angel gene", and the "master watchman", referring to its role in conserving stability by preventing genome mutation.

The p53 tumour suppressor gene is mutated in approximately 70 to 80% of colorectal cancer[1,25]. Mutation or loss of p53 usually occurs at the time of the transition from adenoma to carcinoma in the adenoma-carcinoma sequence. The frequency of p53 abnormalities increases with the progression of the lesion. The protein product of p53 gene induces G1 cell-cycle arrest to facilitate DNA repair during replication of cells exposed to environmental or oncogenic stress.

When DNA damage is too great to be repaired, it can induce apoptosis and this is considered a major pathway whereby p53 exerts its tumour suppressor function. Mutation of the p53 tumor suppressor gene is thought to play an important role in the progression of colorectal cancer and might therefore represent a clinically useful marker of prognosis.

Despite 20 years of investigation, the impact of p53 mutation on clinical outcome is far from being conclusive. Prognostication of newly diagnosed colorectal cancer (CRC) predominantly relies on stage as defined by the American Joint Committee on Cancer classification. The 5-year survival rate for patients with CRC is largely dependent on TNM stage[26]. The TNM staging system was initially developed to predict prognosis, but its function has expanded to aid in the choice of treatment and in the selection of patients for clinical trials[26].

Tumour extent, lymph node status, tumour grade and the assessment of lymphatic and venous invasion are still the most important morphological prognostic factors. Evidence suggests that tumour budding and tumour border configuration are important additional histological parameters but are not regarded as essential in prognosis. Although several molecular features, such as LOH18q and p53 mutation analysis have shown promising results in terms of their prognostic value, the American Society of Clinical Oncology Tumor Markers Expert Panel does not currently recommend their use in routine practice[26].

Immunohistochemical markers currently being investigated for their prognostic impact include proteins involved in the wide array of signaling pathways mediating colorectal tumour progression and metastasis.

The WNT signaling pathway involving beta-catenin,[27,28] APC gene[28] & E-cadherin[28,29], the transforming growth factor-(TGF)-beta pathway,[30] the TGF beta receptors TGFbRI, TGFb RII,[31,32] the RAS signaling pathway involving, among the novel potential markers, Raf-1 kinase inhibitor protein (RKIP) [33,34,35] and the receptor for hyaluronic acid mediated motility (RHAMM)[36] are examples of potential targets for immunohistochemical prognostic studies.

Markers of cell cycle arrest (p21, p27, p53[37,38,39]), apoptosis (Bcl-2[40], Bax[41], apoptosis protease activating factor (APAF)-1[42]), proliferation (Ki67[43]) and DNA repair (thymidylate synthase, TS[43]) have also been explored for their prognostic value.

Martin Kruschewski et al[44], studied colorectal adenocarcinomas & the p53 immunohistochemical staining was scored based on the percentage of positive tumour cells as follows:

SCORE	% OF POSITIVE TUMOUR CELLS
0	0%
1	1% - 10%
2	11% - 25%
3	>25%

Antonio Russo et al[45] in his study, which involved a total of 3,583 CRC patients from 25 different research groups in 17 countries, found that the overall frequency of p53 mutation in this CRC series was 42%. 34% of the proximal colon tumours and 45% of the distal colon and rectal tumours had p53 mutations. They were associated with lymphatic invasion in proximal tumours. In distal colon tumours, deletions causing loss of amino acids were associated with worse survival. For proximal colon tumours, only p53 mutations in exon 5 were significantly associated with worse survival. In this group, worse outcome compared with tumours with wild-type p53 was observed for denaturing mutations, multiple mutations, or mutations yielding the same amino acid side group or an amino acid loss. For rectal tumours, only those giving rise to an amino acid loss were significantly associated with worse survival.

A.Conlin et al[46] studied a series of 107 in-patients treated surgically for colorectal cancer and found that patients with K-ras mutations had significantly poorer overall survival than patients without K-ras mutations. K-ras mutations were also significantly associated with poorer disease specific survival. The presence of APC and p53 mutations did not affect survival in this cohort of patients.

Yamaguchi et al[47] studied p53 immunoreactivity in 100 patients with colorectal adenocarcinoma using immunohistochemistry by the use of a monoclonal antibody with a follow-up of up to 4 years. The author found that the immunoreactivity was found in 61% of specimens from 100 patients with colorectal cancer. The pattern of p53 expression was mainly detected in the nuclei of the cancer cells. There was no significant correlation between the expression of p53 and the histologic grade, tumour size, serosal invasion, lymphatic invasion, venous invasion, lymph node metastasis, or liver metastasis.

However, patients with p53-positive tumours had a greater relative risk of death compared with those with p53-negative tumours. The p53-negative tumours showed a recurrence rate of 5.9%. For the p53 positive-tumours, a recurrence rate of 23.8% was recorded. With these results the author concluded that the immunoreactivity of p53 may be a biologic marker of prognostic significance. Similar results have been obtained using direct sequencing to detect p53 mutation.

Sun et al[48] studied the prognostic significance in 293 colorectal adenocarcinoma patients using p53 expression in relation to DNA ploidy. The author found that the nuclear p53 expression showed no relation with survival or Dukes' stage of the tumour. However, the frequency of cytoplasmic expression increased with advancing Dukes' stage and cytoplasmic expression was associated with poor survival. Among tumours of Dukes' stage A to C, cytoplasmic expression showed prognostic value independent of nuclear staining, grade of the tumour, and Dukes' stage. The author concluded that cytoplasmic expression of p53 may be a useful biological indicator of prognosis in colorectal adenocarcinoma. Subsequent analysis conducted in the same patient cohort after a longer follow-up period concluded that positivity for both nuclear and cytoplasmic p53 was associated with the poorest survival.

Zhang et al[49] studied p53 immunoreactivity in 293 patients by immunohistochemistry using CM1, PAb1801, DO7, and DO1 antibodies on paraffin-embedded colorectal adenocarcinomas. The author found that p53-positive staining by any of the antibodies predicted significantly poor prognosis compared with p53-negative reactivity. These results suggest that there was essentially no difference in the

significance of p53 overexpression as detected by any of the four antibodies with regard to clinicopathological variables.

Soong et al[50] studied p53 mutation and expression by both IHC and by polymerase chain reaction single-strand conformational polymorphism (SSCP) respectively which showed accumulation of p53 protein to be associated with improved survival, independent of tumour stage and grade. Mutation of the p53 gene was also associated with a trend towards improved survival, particularly in distal colorectal tumours.

Hamelin et al[51] performed a study which included 85 colorectal carcinomas for mutations in exons 5-8 of this gene. A strong correlation between the presence of a mutation and short survival was observed when tumours were classified according to their histological stage. A multivariate Cox model analysis showed that p53 mutation, rather than 17p allelic loss (previously proposed to convey prognostic information), was retained as the only independent prognostic factor.

Soong et al[52] studied the prognostic significance of p53 gene mutation and influence of tumour site, stage, adjuvant chemotherapy and type of mutation in 995 Dukes' B and C CRC patients, the majority of whom did not receive chemotherapy. The author found that p53 gene mutation had no prognostic value in the overall series or in different site or stage subgroups. None of the different types of p53 gene mutation showed prognostic value. A trend for association with worse survival was observed in the patient subgroup that received adjuvant chemotherapy.

Flamini et al[53] studied prognostic significance of p53 overexpression by immunohistochemistry in 96 consecutive colorectal cancer patients. The author found that forty-seven per cent of the cases were p53 positive, a statistically significant correlation being found with Dukes' stage. In his study advanced Dukes' stage tumours were localized in the right colon, where a higher percentage of p53 positivity (67% versus 40% of the left side), as well as a higher frequency of cytoplasmic staining was observed. The author concluded from the data obtained that there is a strong correlation between p53 nuclear staining and prognosis.

Yuan-Tzu Lan et al[54] studied the value of p53 protein accumulation as a prognostic marker in 258 patients who received surgical treatment for colorectal cancer. The author evaluated p53 expression in tumour tissue by immunohistochemical analysis using the human p53-specific mouse monoclonal antibody, PAb1801. He found that 97 cases (37.6%) had overexpression of p53 in tumour tissues. The accumulation of p53 protein in tumour tissues did not correlate with age, gender, preoperative serum carcinoembryonic antigen (CEA) level, mucin content, nodal status, and tumour stage. A statistically significant correlation was found between p53 overexpression and location of the tumor in the rectum. Well to moderately differentiated tumours had significantly higher frequency of p53 overexpression than poorly differentiated tumors (60 vs. 40%). The author concluded that the accumulation of p53 protein might have a favorable prognostic value in colorectal cancer.

Bouzourene et al[55] has evaluated p53 and K-ras as prognostic factors for Dukes' stage B colorectal cancer in a clinically and therapeutically homogeneous group of 122 sporadic Dukes' B colorectal carcinomas with a median follow-up of 67 months. On

paraffin embedded tissue p53 staining was performed by immunohistochemistry using the monoclonal antibody DO7. Mutations in exons 5-8 of the p53 gene were assayed in paraffin -embedded tissue by the single-strand conformation polymorphism (SSCP) assay. Nuclear p53 staining was found in 57 (47%) tumours. Aberrant migration patterns indicating mutation of the p53 gene were found in 39 (32%) tumours. The author concluded that assessment of p53 protein expression is more discriminative than p53 mutation to predict the outcome of Dukes' stage B tumours.

Heide et al[56] analysed 33 liver metastases of colorectal carcinomas and 19 primary colon carcinomas from the same hospital with respect to mutational changes, loss of heterozygosity and expression of the p53 tumour suppressor gene. Direct sequencing of PCR products corresponding to the coding region of p53 revealed that 13 of 19 primary tumours (68%) and 23 of 33 liver metastases (70%) had mutations in the p53 gene. The distribution of mutations along the coding region of p53 was similar in liver metastases as compared to the primary tumours. Thus, codon specificity did not seem to be a relevant factor and cells carrying specific p53 mutations seem to have no selective advantage in the metastasizing process. Comparing this data with the mutational spectra found in other countries did not reveal differences in the distribution of mutations along the coding region. Most of the metastases analyzed showed loss of heterozygosity (LOH, 9 of 12 cases, 75%) and strong nuclear staining in immunohistochemistry (10 of 17 cases, 59%). In conclusion, this data indicate a mutational rate of 68% in advanced primary colorectal tumours and of 70% in distant hepatic metastases. The data do not directly support a prominent role of p53 in late stages of colorectal tumour dissemination. However, the primaries analysed in this study were predominantly in advanced tumour

stages. Taken together, p53 mutations are frequent events in the progression of colorectal cancer and may enhance the development of distant metastases.

Ann Forslund et al[57] conducted a study in which twenty-nine potentially cured patients with colorectal carcinoma, without recurrent disease for more than 6 years after their primary surgery, were selected to match a group of 41 colorectal cancer patients with early metastatic spread to the liver. All patients were screened for mutations in the p53 gene, exons 5 to 9, by denaturing gradient gel electrophoresis and subsequent sequencing. The frequency of p53 mutations was significantly different in cured patients (60%) compared with patients with early relapse (41%, $P < .05$). A significant difference was found in the distribution of mutations, indicating that potentially cured patients had a different proportion of mutations in conserved regions of p53 (P 5 .02). This difference was explained by a significantly different frequency of mutations in exon 8 (40% v 15%, P 5 .03), which is part of the conserved region V. All mutations in region V were codon 273 mutations in cured patients, whereas three of four mutations were located in codon 273 in patients with metastatic disease. Allelic loss of p53 (loss of heterozygosity [LOH]) was demonstrated in 26% of the cured patients and in 39% of patients with metastatic disease. The combination of mutation and LOH of p53 was the same (17%) in both groups. This indicate that a large number of p53 mutations in colorectal cancer do not promote disease progression. Some mutations, particularly within conserved regions, may even counteract negative functional effects of other p53 structural alterations.

George E. Theodoropoulos et al[58] in his study collected paraffin-embedded materials retrospectively from 164 colorectal adenocarinoma (50 rectal) patients. The median follow-up was 5 years (range: 1 to 14). EGFR (Epidermal Growth Factor

Receptor) and p53 expression were evaluated by immunohistochemistry. Positive p53 immunostaining and EGFR expression was observed in 63.4% and 43.9% of patients, respectively. EGFR and p53 positivity rates were significantly interrelated (p=0.004). No significant correlation was found with the examined clinicopathological parameters except for advanced T-stage, which demonstrated significant associations with p53 expression (p=0.004), EGFR expression (p=0.0001) and p53/EGFR co expression (p=0.001). Hence the author concluded that overexpression of p53 and EGFR in the colorectal cancer patient population is significantly associated with advanced T stage.

C Hanski et al[59] in his study examined human invasive colorectal carcinomas for the overexpression of p53 oncoprotein with the avidin-biotin complex-peroxidase staining procedure which detects p53 protein in paraffin-embedded material. The tumours were categorized as mucinous (22 cases), most of which originated from adenomas, and nonmucinous, which were subdivided into carcinomas originating from adenoma-carcinoma sequence (ACS) (29 cases) and de novo (DN) carcinomas (25 cases). Nineteen DN carcinomas (76%), 21 ACS carcinomas (72%), and 8 mucinous carcinomas (36%) exhibited detectable amounts of p53 protein in the tumour cell nuclei. Strong overexpression of p53 protein coincided with a high percentage 40%) of stained nuclei in 40% of ACS and 48% of DN carcinomas versus 9% of mucinous tumours. The percentage of stained nuclei, intensity of staining, and distribution of the stained areas did not correlate with the grade or the invasive edge of the tumours. These data indicate that mucinous carcinomas differ from non-mucinous colorectal carcinomas in their genetic lesions.

Satoshi Ikeda et al[60] analysed 813 samples from patients with colorectal non-mucinous adenocarcinoma (NMC) and 41 samples from those with colorectal mucinous adenocarcinomas (MC) over a period of 19 years for p53 expression and beta-catenin expression by immunohistochemistry. The overall survival rates of the 41 patients with colorectal MC and the 813 patients with colorectal NMC were compared by Kaplan-Meier survival analysis. The overall 5-year survival rate of patients with colorectal MC was 43.1%, and that of patients with colorectal NMC was 79.4% (P = 0.0001). The frequency of alterations in p53 in colorectal MC was lower than that in NMC, (31.6% and 63.6%, respectively) suggesting that the mucinous type occurs through a different pathway from that through which NMC occurs.

Thus from the above studies it is understood that the value of p53 overexpression as a prognostic marker in colorectal adenocarcinoma is controversial even though most of the studies show poor prognosis in patients with overexpression of p53. Possible causes for such discrepancy include differences in study methods, types of mutations analysed, laboratory techniques, variable duration of follow-up, statistical differences in study power, and heterogeneity in study populations.

P53 mutation is a late event in the adenoma-carcinoma sequence in colorectal carcinogenesis. It is seen in 70% to 80% of colorectal adenocarcinomas[1]. There is a strong correlation between the p53 expression and the stage of the tumour as evidenced by the above studies but the correlation with histological grading has not been clearly defined.

In this study the expression of p53 in varying stages and histological grades of colorectal adenocarcinomas in colectomy specimens at the Department of Pathology, Stanley medical college has been studied. The results of this study were then compared with the above studies.

IMMUNO HISTOCHEMISTRY

IMMUNOHISTOCHEMISTRY

Immunohistochemistry involves two disciplines – immunology and histology. Immunohistochemistry is used to determine expression of particular antigen and its microanatomic location in the tissue. IHC uses antibodies to distinguish the antigenic differences between the cells. These differences can specifically identify the lineage of cell population and define biologically distinct populations of cells within the same lineage.

Immunohistochemistry started in 1940 when Coons developed an immunofluorescence technique to detect corresponding antigen in frozen sections.

Taylor and colleagues in 1974 showed it was possible to demonstrate antigens in routinely processed tissues. Antigen retrieval technique was introduced by Shi and associates in 1991. Antigen retrieval technique is a simple method that involves heating paraffin processed sections at high temperature before IHC staining.

The use of antibody in IHC depends on the sensitivity and specificity of the antigen – antibody reaction and the hybridoma technique provides limitless source of highly specific antibodies.

BLOCKING NON – SPECIFIC BACKGROUND STAINING

Background staining is due to either non specific binding or presence of endogenous enzymes. Non specific binding with polyclonal primary antibody is

minimized by pre incubating sections with serum from same species on optimal working dilution.

Endogenous enzymes such as peroxidase seen in normal and neoplastic tissues is abolished by peroxidase blocking or by using alternate systems such as immunogold technique.

Methods suggested to overcome endogenous peroxidase activity include incubation in methanol containing 0.5% hydrogen peroxide for 10 minutes at room temperature (almost complete abolition of endogenous peroxidase activity). Endogenous alkaline phosphatase is blocked by addition of 0.1 M concentration of levamisole to the enzyme substrate solution.

DETECTION SYSTEMS

Antibodies are labeled or flagged by some method to permit visualization – these include fluorescent substances, enzymes forming colored reaction with suitable substrate (light microscopy) or heavy metals (electron microscopy).

METHODS OF IHC

DIRECT LABELING METHOD

Antibody is attached with a label by chemical means and directly applied to tissue sections. It is a rapid and easy procedure and carries the disadvantage of multiple antigens which require separate incubation with respective antibodies.

INDIRECT LABELING METHOD

Enzymes are labeled with the secondary antibody, which is produced against the primary antibody. This method is more sensitive and easy to handle. The advantages also include increased versatility, higher working dilution of primary antibody, secondary antibodies against primary antibodies of different species and ease of preparation.

AVIDIN BIOTIN TECHNIQUES

High affinity binding between biotin and avidin is used in this procedure. Biotin is chemically linked to primary antibody and avidin is conjugated chemically to enzyme. The avidin binds to biotinylated antibody thus localizing the peroxidase moiety at the site of antigen.

Disadvantages of this technique are that the endogenous biotin produces non specific background staining.

AVIDIN BIOTIN CONJUGATE PROCEDURE

In this technique primary antibody is added followed by biotinylated secondary antibody and next by preformed complexes of avidin and biotin horse raddish peroxidase conjugate. This is a more sensitive method.

BIOTIN STREPTAVIDIN SYSTEM

Streptavidin is used in place of avidin. Streptavidin complexes are more stable.

IMMUNOGOLD SILVER STAINING TECHNIQUE

This is used in ultrastuctural immunolocalisation. Gold particles are enhanced by the addition of several layers of metallic silver. The fine silver deposits in the background create confusion when small amounts of antigen are identified.

POLYMERIC METHOD

This technique permits binding of large number of enzyme molecules to a secondary antibody via the dextran backbone. Advantages of this technique are increased sensitivity, minimized non specific background staining and a reduction in the total number of assay steps.

TISSUE FIXATION, PROCESSING AND ANTIGEN RETRIEVAL TECHNIQUES

Tissues for IHC undergo fixation, dehydration and paraffin embedding.

FIXATION

This is a critical step as the preservation of morphology is essential for interpretation of IHC. 10% buffered neutral formalin is commonly used because of the following advantages.

1. Good morphological preservation
2. Cheap

3. Sterilizes tissues

4. Carbohydrate antigens are better preserved.

The disadvantage of masking of antigens during fixation can be overcome by antigen retrieval techniques.

ANTIGEN RETRIEVAL

This procedure involves unmasking of the antigens. Following techniques can be used.

1. Proteolytic enzyme digestion

2. Microwave antigen retrieval

3. Microwave and trypsin antigen retrieval technique

4. Pressure cooker antigen retrieval

MATERIALS AND METHODS

MATERIALS AND METHODS

STUDY DESIGN: - Longitudinal retrospective study

TIME PERIOD: - July 2009 to August 2011.

SETTING: - Department of Pathology, Stanley medical college, Chennai.

SAMPLE: - A total of 120 colectomy specimens received from the departments of General Surgery and Surgical Gastroenterology.

PLAN

The patients who were included in this study were screened with predetermined inclusion and exclusion criteria. Selected patients underwent through consent protocols. Brief clinical history and examination findings were collected with predetermined proforma.

INCLUSION CRITERIA

All cases proved to be adenocarcinomas of colorectal region by histopathology irrespective of age and sex were included for the study.

EXCLUSION CRITERIA

All malignancies of the colorectal region other than adenocarcinomas and those cases with poor clinical data were excluded from the study.

METHOD OF DATA COLLECTION

All the cases enrolled in the study according to the above criteria were evaluated. Of the 120 cases, adenocarcinomas found in 115 cases. Among the 115 cases 100 cases had adequate clinical and investigatory data. Clinical history and colonoscopy findings were tabulated. Adequate samples were taken from the growths in these specimens.

The tissues so obtained were processed and sections were cut at 5 microns. Hematoxylin and eosin staining of the sections were done and histopathological appearance and extent of the malignancies were studied. Necessary microphotographs were taken.

The adenocarcinomas found have been categorized into well differentiated, moderately differentiated and poorly differentiated based on the amount of glandular architecture. The number of cases in each category was tabulated.

The staging of the malignancy was done according to the TNM staging system and the results were tabulated.

Immunohistochemical study of p53 expression was done in 50 cases and the degree of p53 expression was scored in each case using the percentage of cells showing p53 positivity. The p53 score was correlated with the TNM stage and histological grade of the colorectal adenocarcinomas. The results were tabulated and conclusion was drawn.

METHODS OF TISSUE PREPARATION FOR IHC

Ten percent buffered formalin was used for fixing the specimens, the tissues were processed in various grades of alcohol and xylene using automated histokinette. Paraffin blocks were prepared and sections of 5 microns thickness were cut in semiautomatic microtome using disposable blades and stained with hematoxylin and eosin. Suitable blocks were chosen for IHC.

Sections for immunohistochemistry were also cut in semiautomatic microtome using disposable blades. Slides were subjected to antigen retrieval using the microwave technique using TRIS EDTA (pH 9.2) buffer solution and then treated by HRP (Horse radish peroxidase) polymer technique.

HRP POLYMER TECHNIQUE

The coated slides were taken through the following stages

1. Treatment with peroxidase block – for inhibiting endogenous peroxidase in the tissue for 20 minutes.
2. Wash in TRIS buffer for 5 minutes.
3. Application of power block – blocks non specific antigen antibody reaction – 20 minutes.
4. Blot dry the excess power block.
5. Application of primary antibody for 60 minutes.
6. Wash in TRIS buffer for 5 minutes thrice.
7. Application of super enhancer for 30 minutes which enhances the final reaction product by increasing the sensitivity of antigen antibody reaction.

8. Application of SS label – secondary antibody from goat with the tagged horse radish peroxidase enzyme for 30 minutes.
9. Wash thrice in TRIS buffer.
10. Application of DAB (Diamino benzidine) chromogen for 5 minutes – this is cleaved by the enzyme to give the coloured product at the antigen sites.
11. Wash in distilled water for 5 minutes.
12. The slides are counterstained with hematoxylin.
13. Air dried and mounted with DPX (Distrene dibutyl pthalide in xylol).

P53 nuclear staining intensity ranged from strong to moderate to weak. Extent of staining varied from multiple positive foci to scattered or rare foci.

METHOD OF SCORING FOR p53

SCORE	% OF POSITIVE TUMOUR CELLS
0	0%
1	1% - 10%
2	11% - 25%
3	>25%

Nuclear p53 positivity was defined as staining of the nucleus, irrespective of the percentage of positive cells.

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

A total of 100 cases were studied. Histopathological examination and TNM staging was done which showed the following results.

ADENOCARCINOMAS - 100

STAGE

I - 32

II - 25

III - 18

IV - 25

GRADE

I (Figure : 1) - 41

II (Figure : 2) - 32

III (Figure : 3) - 20

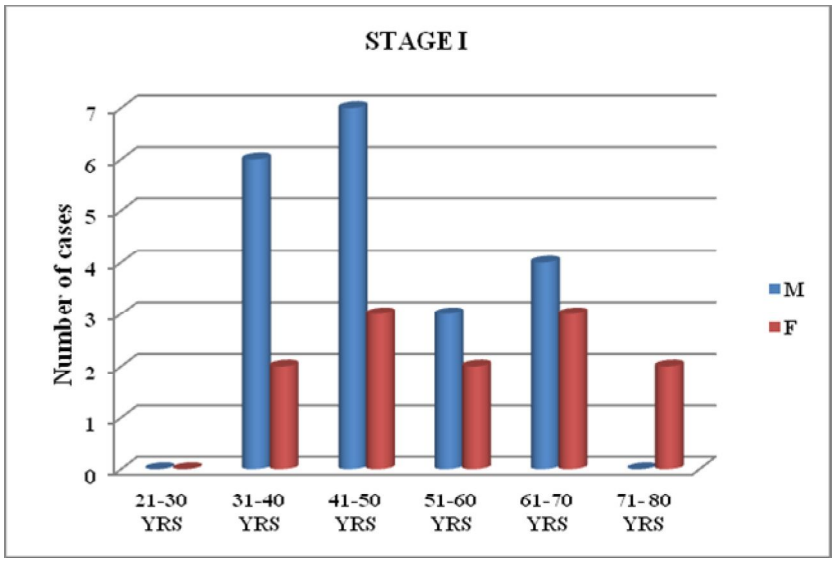
Mucinous carcinoma (Figure : 4) - 07

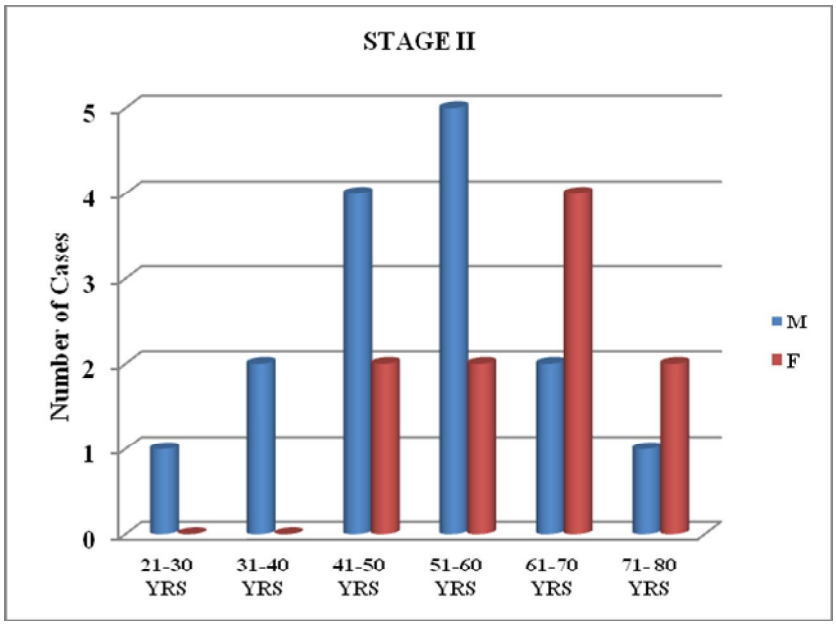
AGE AND SEX DISTRIBUTION OF PATIENTS WITH COLORECTAL ADENOCARCINOMAS IN RELATION TO STAGE

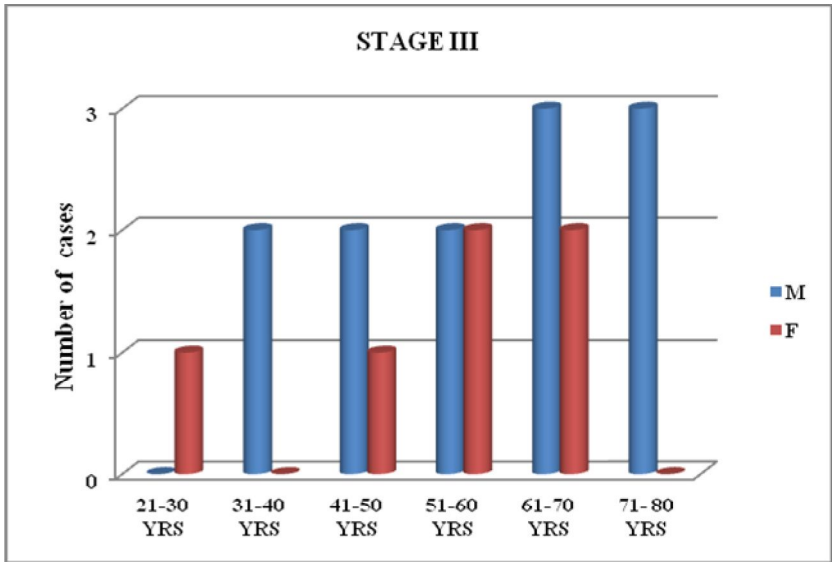
TABLE NO 1

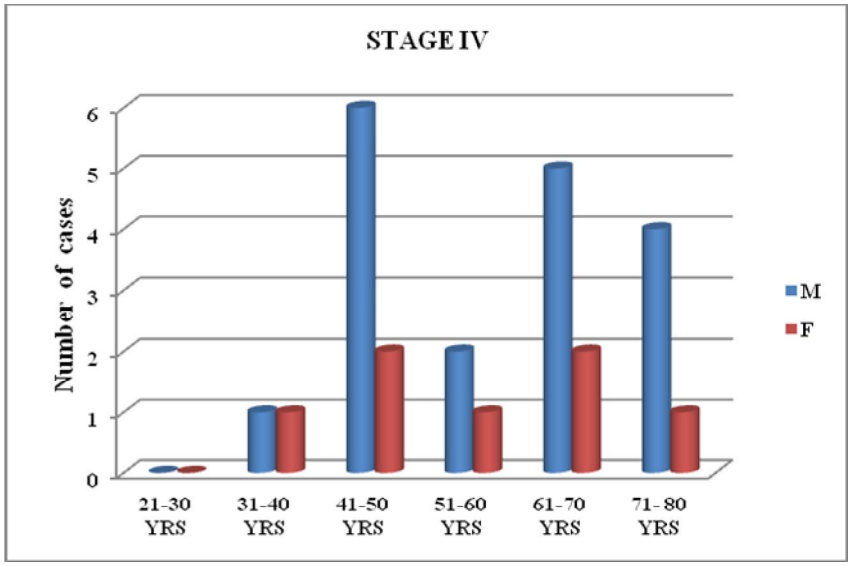
AGE	STAGE I			STAGE II			STAGE III			STAGE IV			TOTAL		
	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T
21 TO 30	0	0	0	1	0	1	0	1	1	0	0	0	1	1	2
31 TO 40	6	2	8	2	0	2	2	0	2	1	1	2	11	3	14
41 TO 50	7	3	10	4	2	6	2	1	3	6	2	8	19	8	27
51 TO 60	3	2	5	5	2	7	2	2	4	2	1	3	12	7	19
61 TO 70	4	3	7	2	4	6	3	2	5	5	2	7	14	11	25
71 TO 80	0	2	2	1	2	3	3	0	3	4	1	5	8	5	13
TOTAL	20	12	32	15	10	25	12	6	18	18	7	25	65	35	100

Age of patients with colorectal adenocarcinoma was ranging from 24 to 78 years and it was more common in males. Stages I & II were categorized as low stage and stages III & IV were categorized as high stage. High stage tumours were common in males. In those above 60 years of age high stage tumors were common.


STAGE I
Number of cases
0
1
2
3
4
5
6
7
21-30 YRS
31-40 YRS
41-50 YRS
51-60 YRS
61-70 YRS
71-80 YRS
M
F


STAGE II
Number of Cases
0
1
2
3
4
5
21-30 YRS
31-40 YRS
41-50 YRS
51-60 YRS
61-70 YRS
71-80 YRS
M
F


STAGE III
Number of cases
0
1
2
3
21-30 YRS
31-40 YRS
41-50 YRS
51-60 YRS
61-70 YRS
71-80 YRS
M
F

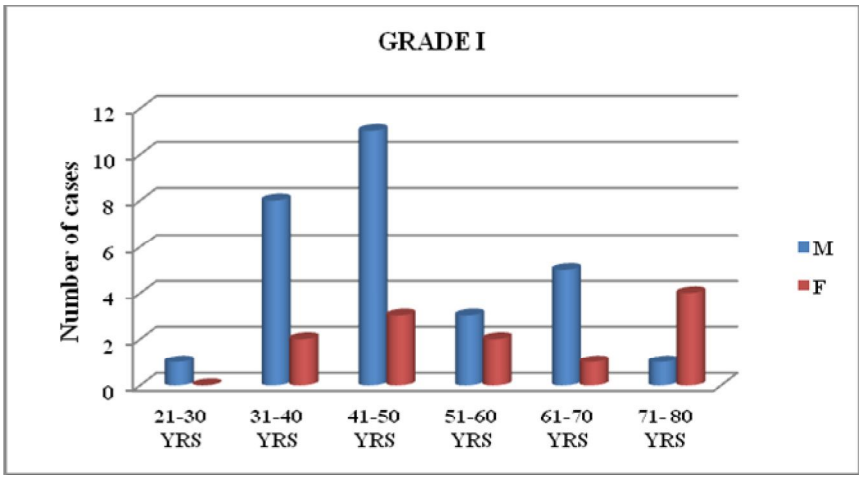

STAGE IV
Number of cases
0
1
2
3
4
5
6
21-30 YRS
31-40 YRS
41-50 YRS
51-60 YRS
61-70 YRS
71-80 YRS
M
F

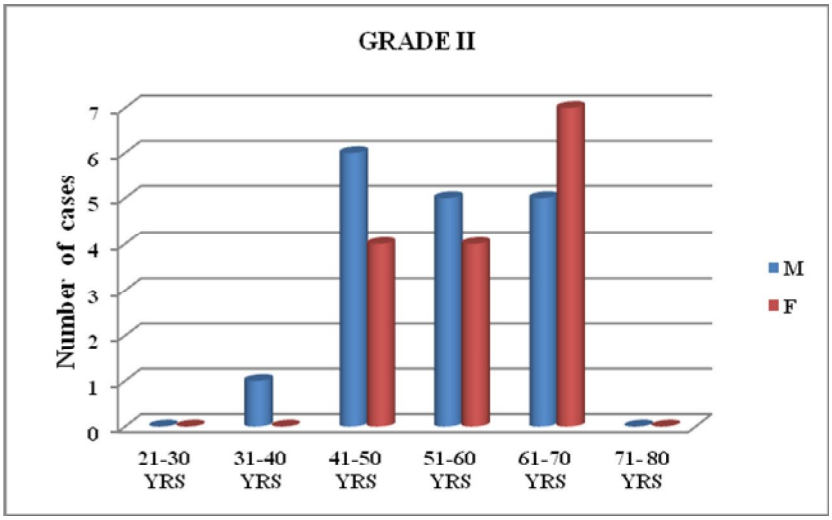
AGE AND SEX DISTRIBUTION OF PATIENTS WITH COLORECTAL ADENOCARCINOMAS IN RELATION TO HISTOLOGICAL GRADE

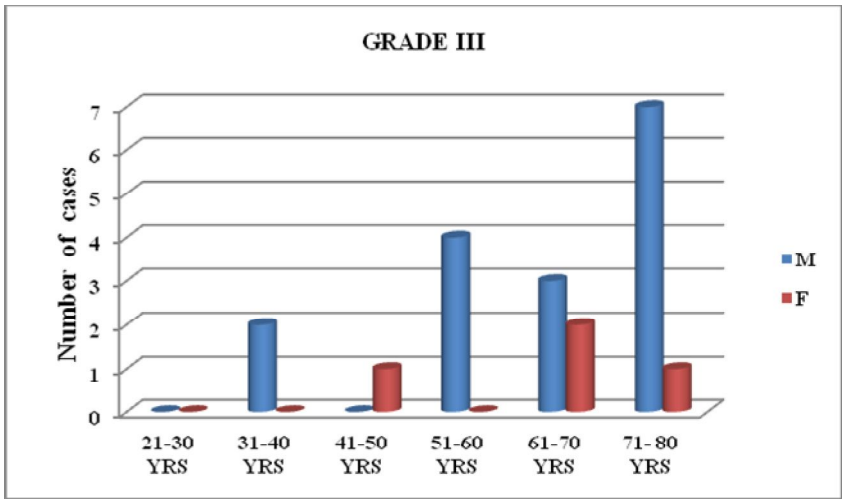
TABLE NO 2

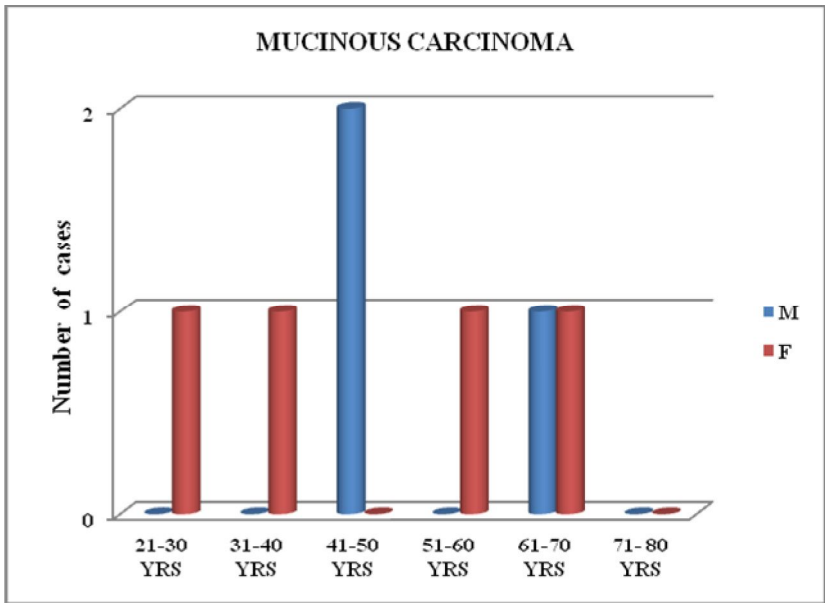
AGE	GRADE I			GRADE II			GRADE III			MUCINOUS CARCINOMA			TOTAL		
	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T
21 TO 30	1	0	1	0	0	0	0	0	0	0	1	1	1	1	2
31 TO 40	8	2	10	1	0	1	2	0	2	0	1	1	11	3	14
41 TO 50	11	3	14	6	4	10	0	1	1	2	0	2	19	8	27
51 TO 60	3	2	5	5	4	9	4	0	4	0	1	1	12	7	19
61 TO 70	5	1	6	5	7	12	3	2	5	1	1	2	14	11	25
71 TO 80	1	4	5	0	0	0	7	1	8	0	0	0	8	5	13
TOTAL	29	12	41	17	15	32	16	4	20	3	4	7	65	35	100

Grade I & II tumours were categorized as low grade and grade III & mucinous carcinomas were categorized as high grade. In this study low grade tumours predominated in both the sexes and the high grade tumours were more common in males. Mucinous carcinomas showed slight female preponderance. However the statistical analysis using Chi-Square test revealed no significant correlation (P value = 0.247) between sex and the grade/histological type of the tumour.


GRADE I
Number of cases
12
10
8
6
4
2
0
21-30 YRS
31-40 YRS
41-50 YRS
51-60 YRS
61-70 YRS
71-80 YRS
M
F


GRADE II
Number of cases
7
6
5
4
3
2
1
0
21-30 YRS
31-40 YRS
41-50 YRS
51-60 YRS
61-70 YRS
71-80 YRS
M
F


GRADE III
Number of cases
0
1
2
3
4
5
6
7
21-30 YRS
31-40 YRS
41-50 YRS
51-60 YRS
61-70 YRS
71-80 YRS
M
F

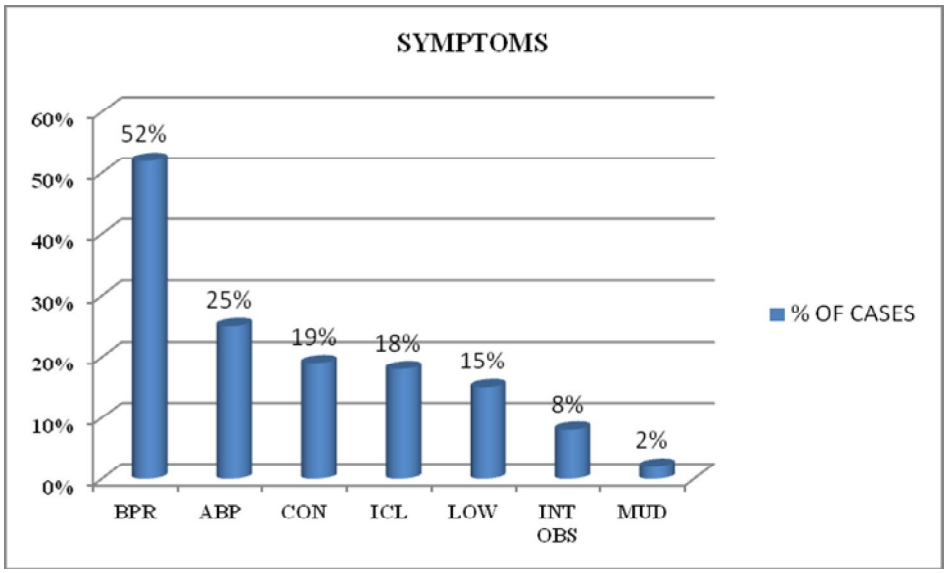

MUCINOUS CARCINOMA
Number of cases
0
1
2
21-30 YRS
31-40 YRS
41-50 YRS
51-60 YRS
61-70 YRS
71-80 YRS
M
F

CLINICAL PRESENTATION OF COLORECTAL ADENOCARCINOMAS

TABLE NO 3

SYMPTOMS	NO OF CASES	%
BLEEDING PER RECTUM	52	52%
ABDOMINAL PAIN	25	25%
CONSTIPATION	19	19%
INTERMITTENT CONSTIPATION & LOOSE STOOLS	18	18%
LOSS OF WEIGHT	15	15%
INTESTINAL OBSTRUCTION	8	8%
MUCOUS DIARRHOEA	2	2%

Most common presenting symptoms of colorectal adenocarcinomas were bleeding per rectum and altered bowel habits.



BPR – Bleeding per rectum

ABP – Abdominal pain

CON – Constipation

ICL – Intermittent constipation and loose stools

LOW – Loss of weight

INT OBS – Intestinal obstruction

MUD – Mucus diarrhoea

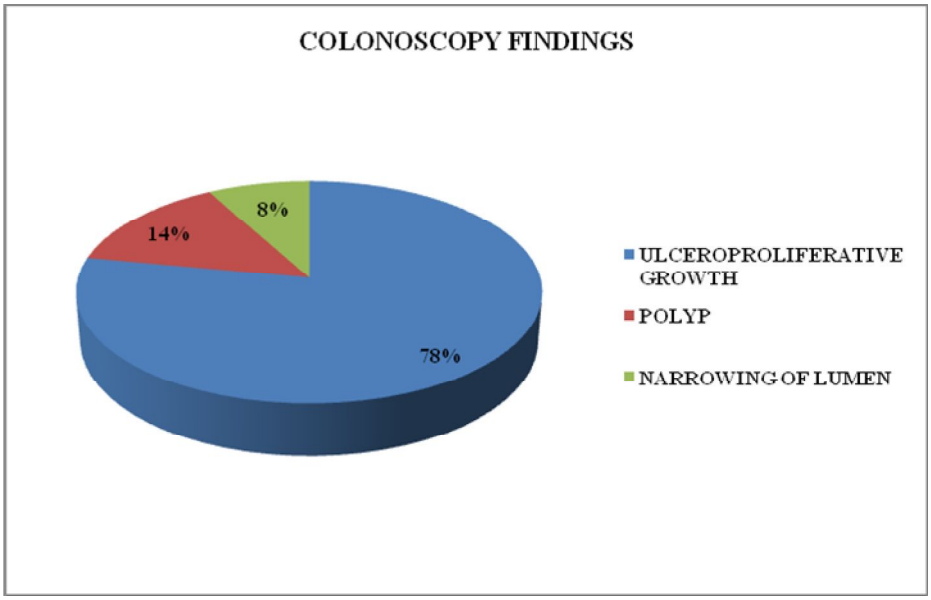
COLONOSCOPIC FINDINGS

COLORECTAL ADENOCARCINOMA

TABLE NO 4

COLONOSCOPIC FINDINGS	NO OF CASES
ULCEROPROLIFERATIVE GROWTH	78
POLYP	14
NARROWING OF LUMEN	8
TOTAL	100

Most of the colorectal adenocarcinomas exhibited an ulceroproliferative growth pattern grossly.


COLONOSCOPY FINDINGS
8%
14%
78%
ULCEROPROLIFERATIVE GROWTH
POLYP
NARROWING OF LUMEN

SITE DISTRIBUTION OF COLORECTAL ADENOCARCINOMAS

TABLE NO 5

SITE	NO OF CASES
CAECUM	18
ASCENDING COLON	14
TRANSVERSE COLON	6
DESCENDING COLON	4
SIGMOID COLON	14
RECTUM	44
TOTAL	100

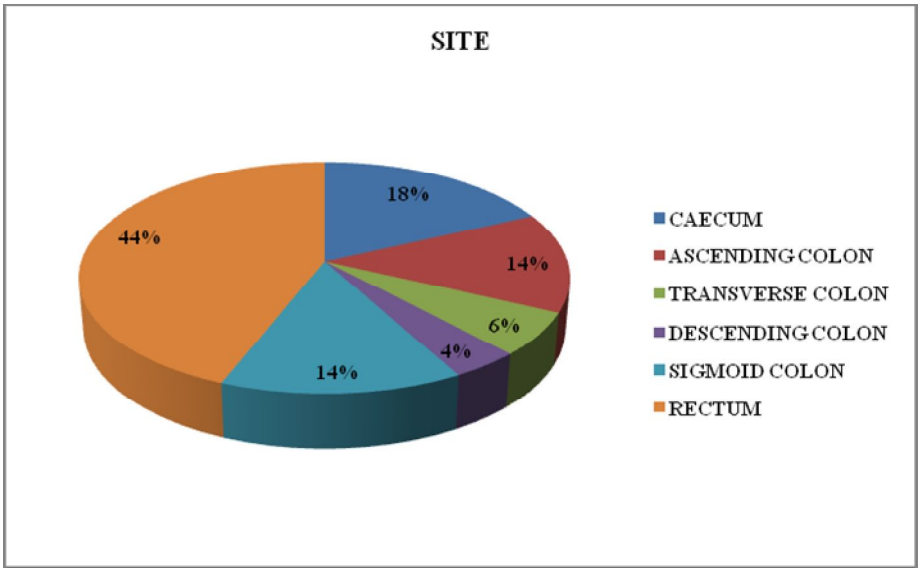
Rectum was the commonest site for colorectal adenocarcinomas (Figures 20 and 21).

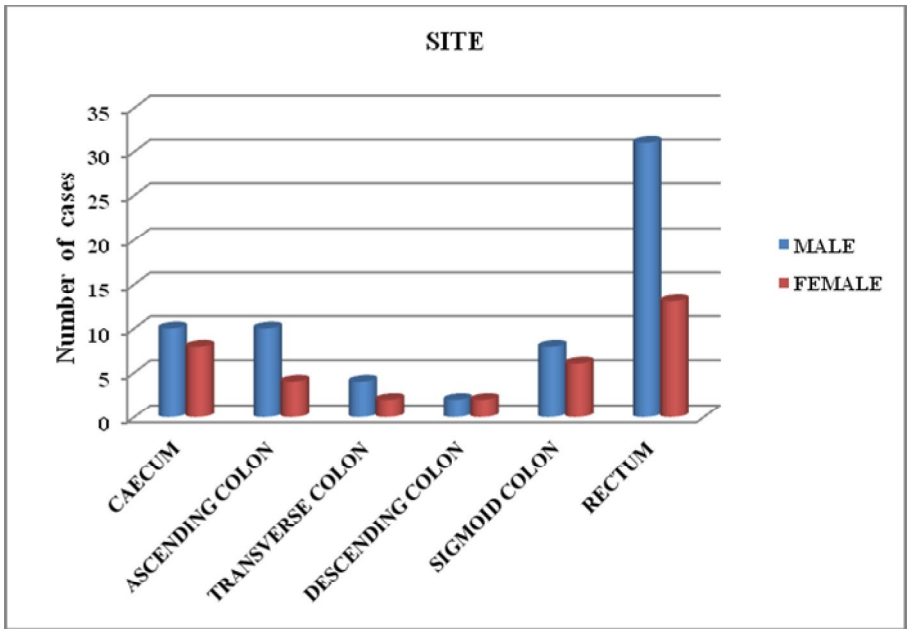
SITE DISTRIBUTION OF COLORECTAL ADENOCARCINOMAS IN RELATION TO SEX

TABLE NO 6

SITE	MALE	FEMALE	TOTAL
CAECUM	10	8	18
ASCENDING COLON	10	4	14
TRANSVERSE COLON	4	2	6
DESCENDING COLON	2	2	4
SIGMOID COLON	8	6	14
RECTUM	31	13	44
TOTAL	65	35	100

Rectum was the commonest site of occurrence of colorectal adenocarcinoma in both the sexes.


SITE
18%
14%
6%
4%
14%
44%
CAECUM
ASCENDING COLON
TRANSVERSE COLON
DESCENDING COLON
SIGMOID COLON
RECTUM


SITE
Number of cases
35
30
25
20
15
10
5
0
CAECUM
ASCENDING COLON
TRANSVERSE COLON
DESCENDING COLON
SIGMOID COLON
RECTUM
MALE
FEMALE

SITE DISTRIBUTION OF COLORECTAL ADENOCARCINOMAS IN RELATION TO AGE

TABLE NO 7

SITE	21-30	31-40	41-50	51-60	61-70	71-80	TOTAL
CAECUM	1	1	5	3	5	3	18
ASCENDING COLON	0	4	4	2	3	1	14
TRANSVERSE COLON	0	1	1	1	2	1	6
DESCENDING COLON	0	0	1	2	0	1	4
SIGMOID COLON	0	2	5	1	5	1	14
RECTUM	1	6	11	10	10	6	44
TOTAL	2	14	27	19	25	13	100

In all the age groups the rectosigmoidal region was the commonest site for adenocarcinomas.

SIZE DISTRIBUTION IN COLORECTAL ADENOCARCINOMAS

TABLE NO 8

SIZE	NO OF CASES
UPTO 5 CM	**62**
6-10 CM	**32**
>10 CM	**6**
TOTAL	**100**

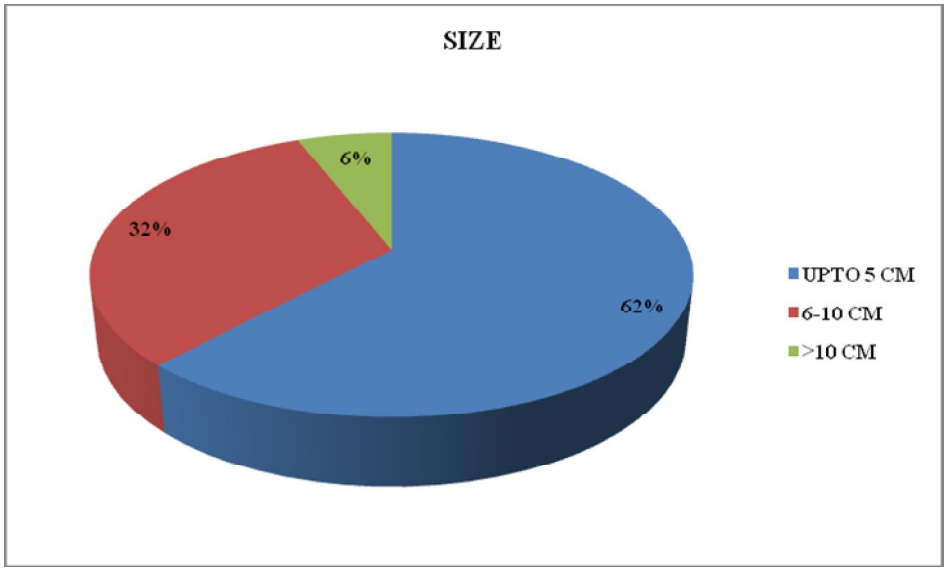
Size of the tumours was ranging from 3 to 13 cm in maximum dimension. Smaller tumours were commonly observed probably due to early diagnosis because of increasing awareness and utilization of medical services.

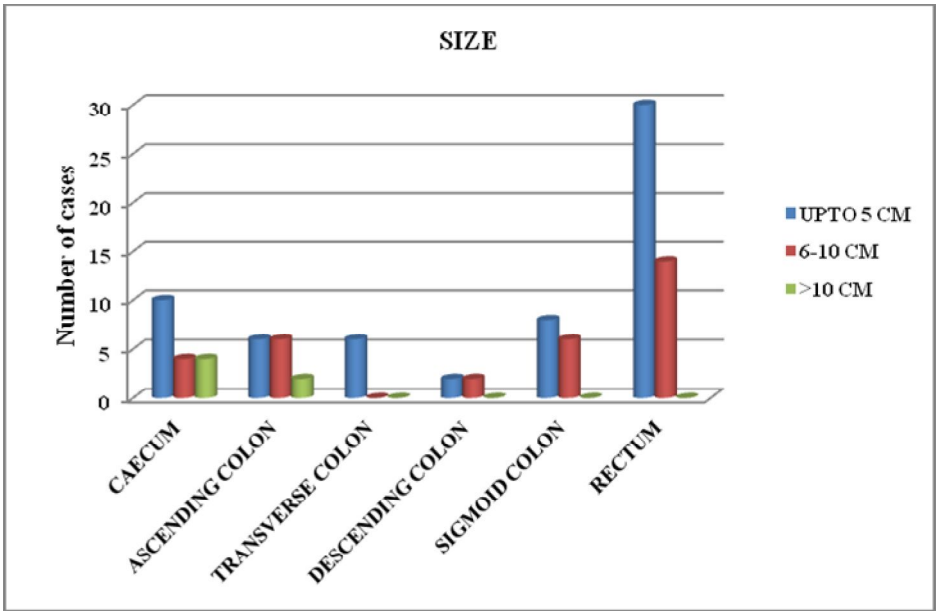
SIZE DISTRIBUTION OF COLORECTAL ADENOCARCINOMAS IN RELATION TO SITE

TABLE NO 9

SITE	SIZE			TOTAL
	UPTO 5 CM	6-10 CM	>10 CM	
CAECUM	10	4	4	18
ASCENDING COLON	6	6	2	14
TRANSVERSE COLON	6	0	0	6
DESCENDING COLON	2	2	0	4
SIGMOID COLON	8	6	0	14
RECTUM	30	14	0	44
TOTAL	62	32	6	100

Larger tumours(>10 cm) were seen commonly in caecum probably due to the larger luminal diameter and late presentation.


SIZE
6%
32%
62%
UPTO 5 CM
6-10 CM
>10 CM


SIZE
Number of cases
30
25
20
15
10
5
0
CAECUM
ASCENDING COLON
TRANSVERSE COLON
DESCENDING COLON
SIGMOID COLON
RECTUM
UPTO 5 CM
6-10 CM
>10 CM

P53 EXPRESSION IN COLORECTAL ADENOCARCINOMAS

TABLE NO 10

S.NO	BIOPSY NO.	HISTOLOGICAL GRADE	STAGE (AJCC)	P53 SCORE
1	3044/09	III	III	3
2	3164/09	III	III	2
3	3241/09	MUCINOUS CARCINOMA	III	0
4	3470/09	I	I	1
5	3900/09	I	I	0
6	4255/09	III	III	2
7	4422/09	I	IV	2
8	4464/09	I	III	2
9	4500/09	I	I	1
10	4501/09	I	I	0
11	4615/09	II	IV	2
12	4616/09	I	I	0
13	4661/09	I	I	0

14	4828/09	II	I	0
15	4879/09	II	IV	3
16	5127/09	III	III	1
17	5420/09	I	IV	3
18	5553/09	I	IV	3
19	5880/09	I	III	2
20	5960/09	II	II	2
21	6004/09	I	I	3
22	6024/09	MUCINOUS CARCINOMA	II	0
23	6079/09	I	I	1
24	6091/09	I	I	1
25	6129/09	I	IV	3
26	758/10	II	II	2
27	2712/10	II	II	1
28	2735/10	I	II	0
29	3052/10	I	IV	3

30	3266/10	II	II	3
31	3276/10	I	IV	2
32	3732/10	I	IV	3
33	3844/10	MUCINOUS CARCINOMA	II	1
34	3879/10	III	II	2
35	4009/10	I	I	3
36	4028/10	II	II	3
37	4183/10	I	I	2
38	4337/10	I	III	3
39	4478/10	MUCINOUS CARCINOMA	I	0
40	4510/10	I	IV	2
41	4691/10	MUCINOUS CARCINOMA	II	3
42	4889/10	I	IV	2
43	4938/10	II	III	0
44	5955/10	I	IV	2
45	6010/10	MUCINOUS CARCINOMA	III	1

46	6115/10	III	IV	3
47	105/11	III	IV	3
48	199/11	II	III	3
49	630/11	III	II	0
50	839/11	III	IV	3

P53 EXPRESSION IN COLORECTAL ADENOCARCINOMAS

TABLE NO 11

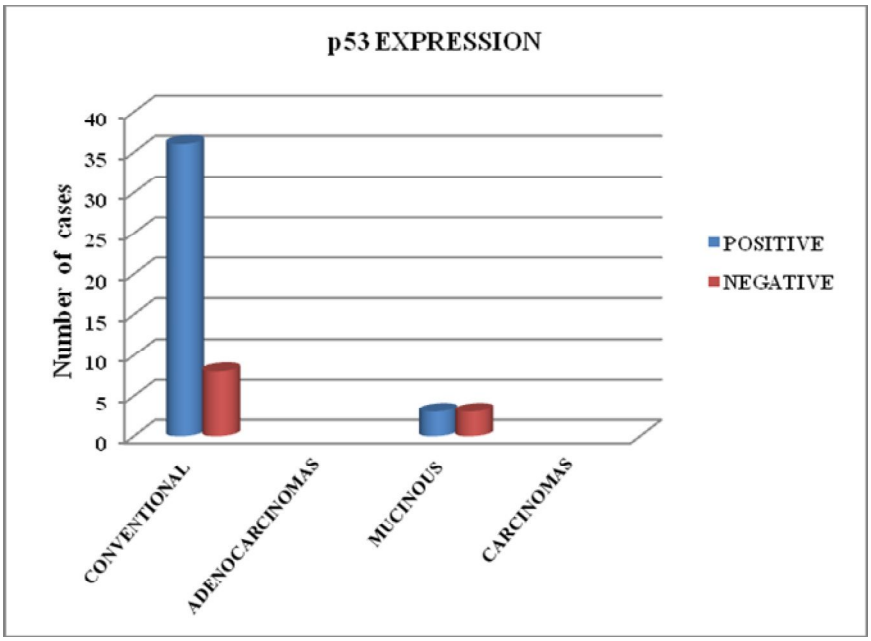
P53 EXPRESSION	POSITIVE	NEGATIVE	TOTAL
CONVENTIONAL ADENOCARCINOMAS	36	8	44
MUCINOUS CARCINOMAS	3	3	6
TOTAL	39	11	50

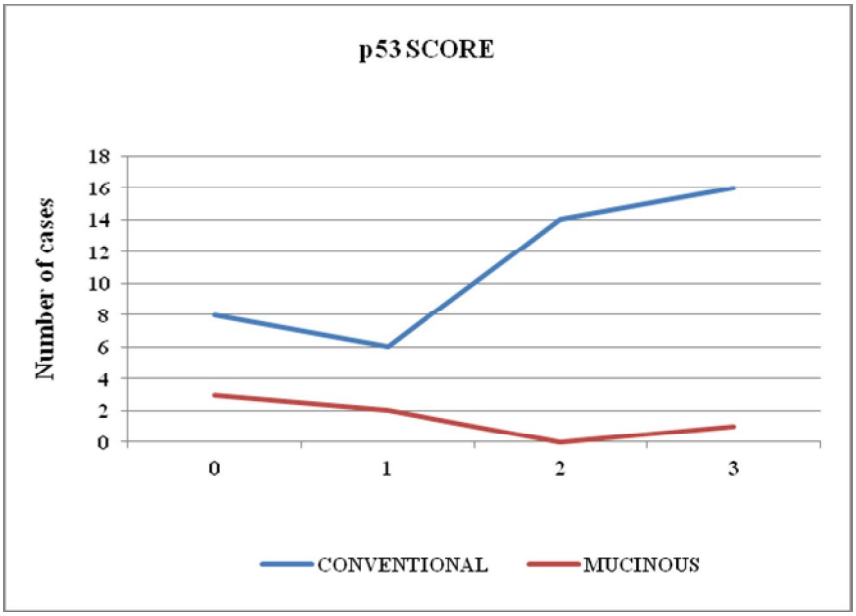
P53 EXPRESSION IN RELATION TO HISTOLOGY

TABLE NO 12

ADENOCARCINOMAS	P53 SCORE				TOTAL
	0	1	2	3	
CONVENTIONAL ADENOCARCINOMAS	8	6	14	16	44
MUCINOUS ADENOCARCINOMAS	3	2	0	1	6
TOTAL	11	8	14	17	50

In this study 78% of the colorectal adenocarcinomas revealed p53 positivity. The mucinous carcinomas showed a lower p53 score but the P value for this finding was 0.101 which is not statistically significant. This may be due to the smaller sample size.


p53 EXPRESSION
Number of cases
40
35
30
25
20
15
10
5
0
CONVENTIONAL
ADENOCARCINOMAS
MUCINOUS
CARCINOMAS
POSITIVE
NEGATIVE


p53 SCORE
Number of cases
18
16
14
12
10
8
6
4
2
0
0
1
2
3
CONVENTIONAL
MUCINOUS

P53 EXPRESSION IN CONVENTIONAL ADENOCARCINOMAS IN RELATION TO STAGE (AJCC)

TABLE NO 13

STAGE (AJCC)	P53 SCORE				TOTAL	%
	0	1	2	3		
I	5	4	1	2	12	27.27%
II	2	1	3	2	8	18.18%
III	1	1	4	3	9	20.45%
IV	0	0	6	9	15	34.1%
TOTAL	8	6	14	16	44	100%

There is progressive increase in p53 score as the stage increases. Statistical analysis using Chi-Square test was done which revealed a P value of 0.027 which is statistically significant.

P53 EXPRESSION IN MUCINOUS CARCINOMAS IN RELATION TO STAGE (AJCC)

TABLE NO 14 `

STAGE (AJCC)	P53 SCORE				TOTAL
	0	1	2	3	
I	1	0	0	0	1
II	1	1	0	1	3
III	1	1	0	0	2
IV	0	0	0	0	0
TOTAL	3	2	0	1	6

Most of the mucinous carcinomas were in low stage and showed lower p53 scores. P value cannot be determined due to smaller sample size.

P53 EXPRESSION IN COLORECTAL ADENOCARCINOMAS IN RELATION TO HISTOLOGICAL GRADE

TABLE NO 15

HISTOLOGICAL GRADE	P53 SCORE				TOTAL	%
	0	1	2	3		
I	5	4	8	8	25	50%
II	2	1	3	4	10	20%
III	1	1	3	4	9	18%
MUCINOUS CARCINOMA	3	2	0	1	6	12%
TOTAL	11	8	14	17	50	100%

In different grades of the tumour p53 score was ranging from 0 to 3 (Figures 5 to 19). Grade III tumours commonly had high scores and most mucinous tumours had low scores. But among the cases with high scores grade I & II tumours have predominated (70.6%). P value was 0.821 which is not significant statistically

P53 EXPRESSION IN CONVENTIONAL ADENOCARCINOMAS IN RELATION TO THE SITE OF THE TUMOUR

TABLE NO 16

SITE	0	1	2	3	TOTAL	%
CAECUM	2	2	2	4	10	20%
ASCENDING COLON	2	4	2	0	8	16%
TRANSVERSE COLON	1	0	2	1	4	8%
DESCENDING COLON	0	0	1	1	2	4%
SIGMOID COLON	1	0	1	1	3	6%
RECTUM	5	2	6	10	23	46%
TOTAL	11	8	14	17	50	100%

High p53 scores were obtained predominantly in left sided tumours. However statistical analysis did not prove this association (P value = 0.541).

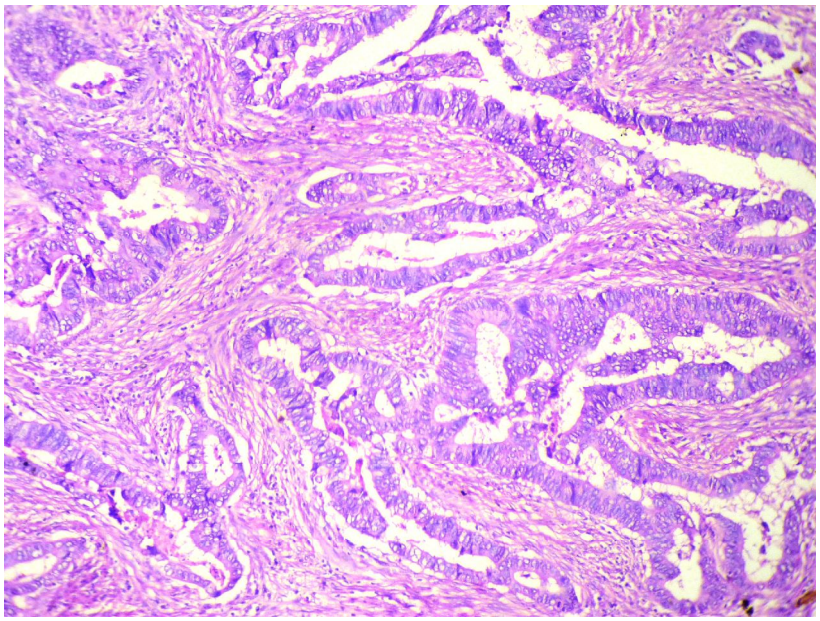


Figure : 1 Well differentiated adenocarcinoma – 10X

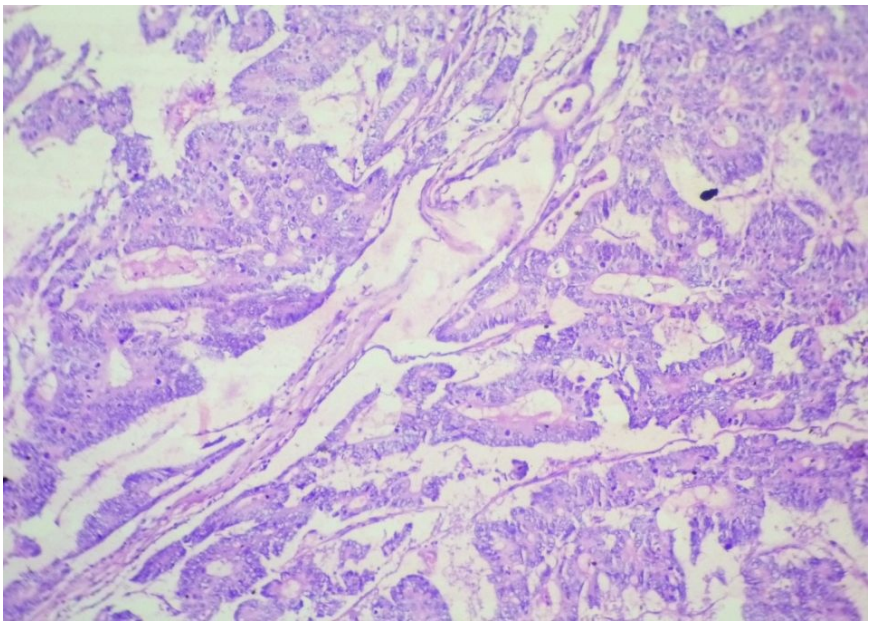


Figure : 2 Moderately differentiated adenocarcinoma – 10X

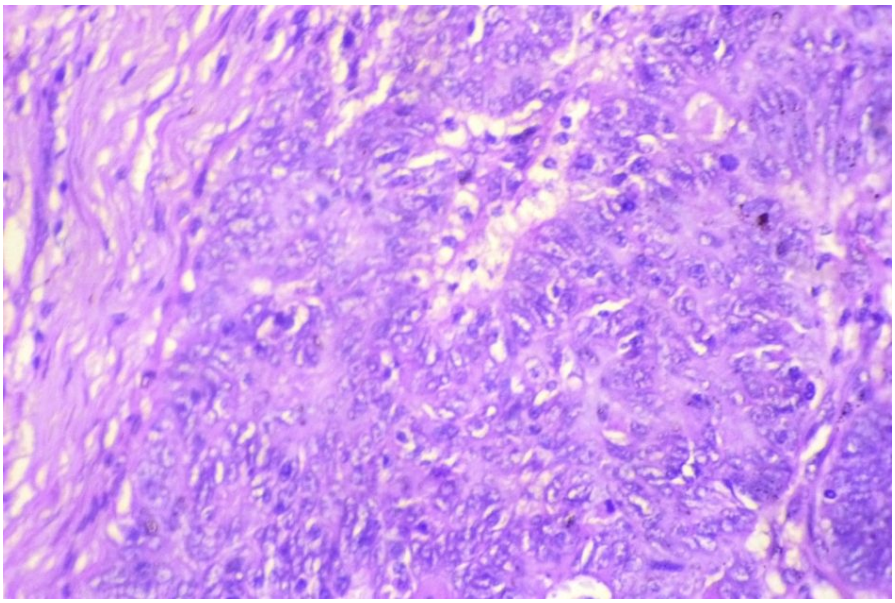


Figure : 3 Poorly differentiated adenocarcinoma – 40X

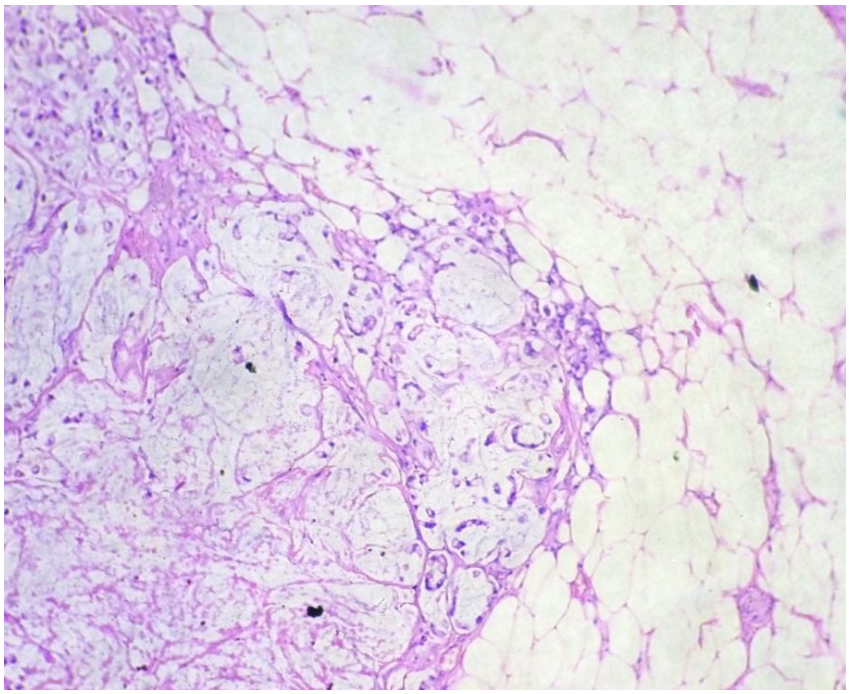


Figure : 4 Mucinous adenocarcinoma – 10X

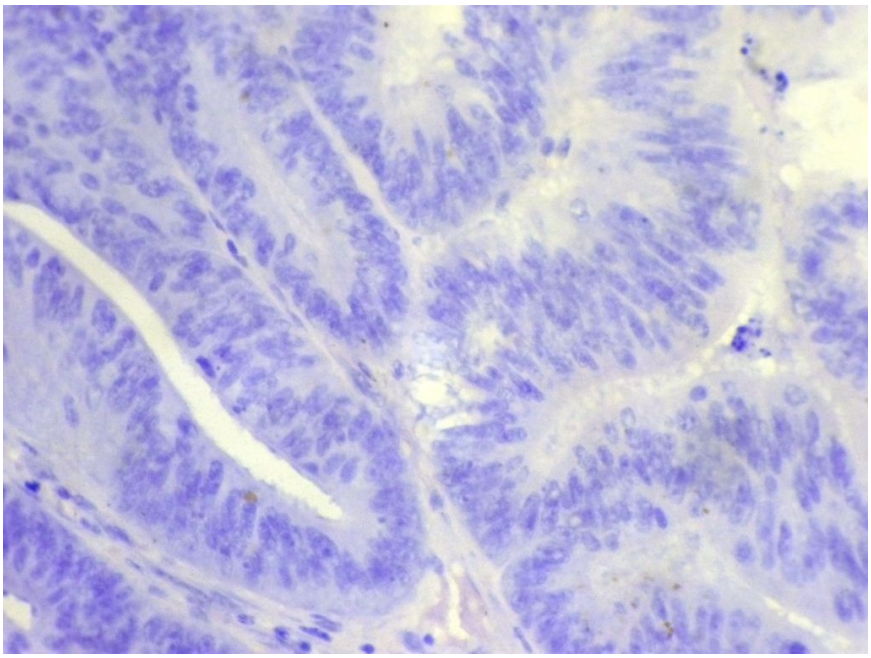


Figure : 5 Well differentiated adenocarcinoma – p53 Score 0

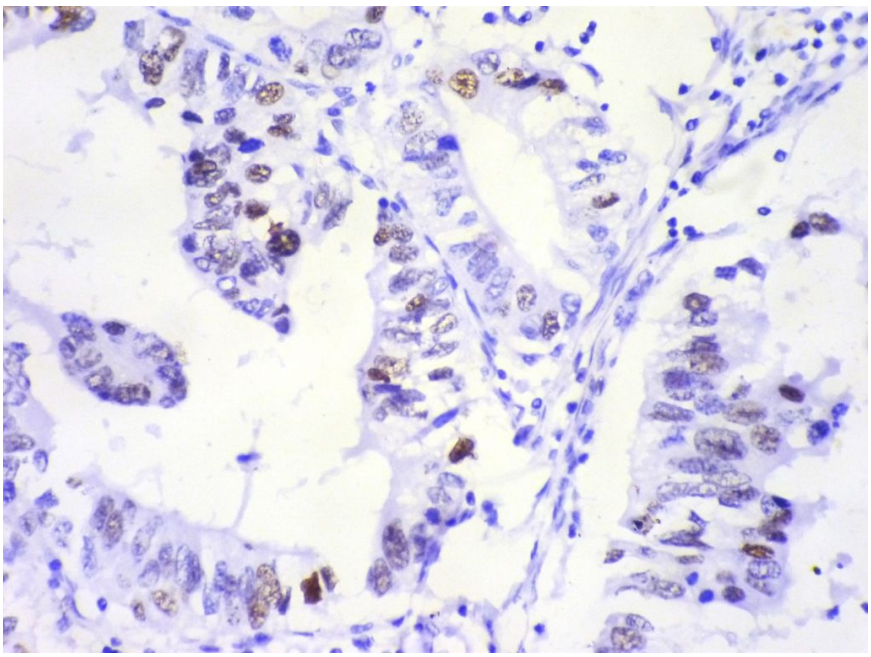


Figure : 6 Well differentiated adenocarcinoma – p53 Score 1

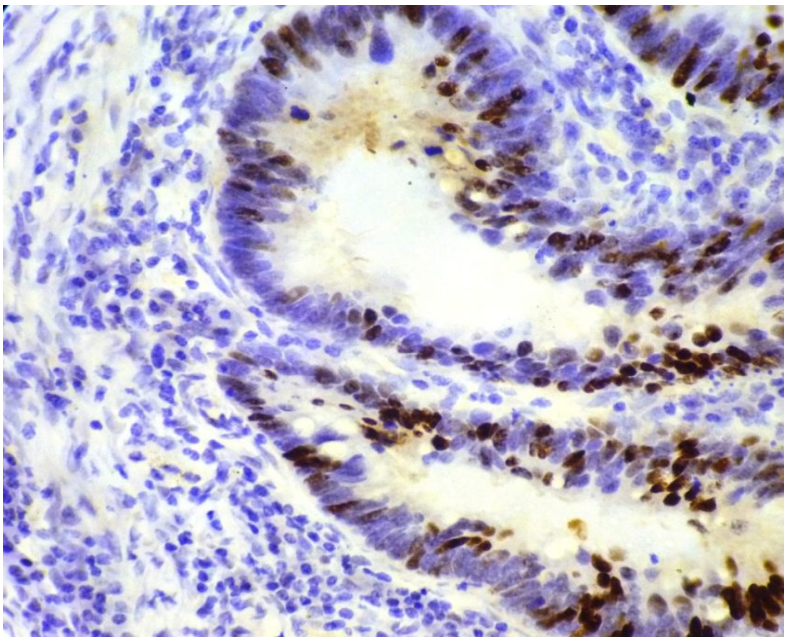


Figure : 7 Well differentiated adenocarcinoma – p53 Score 2

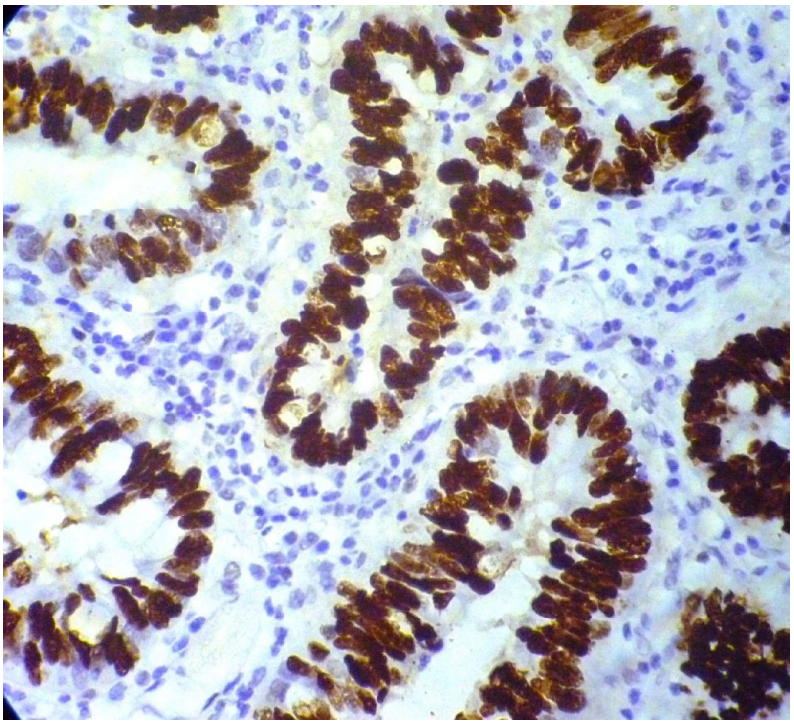


Figure : 8 Well differentiated adenocarcinoma – p53 Score 3

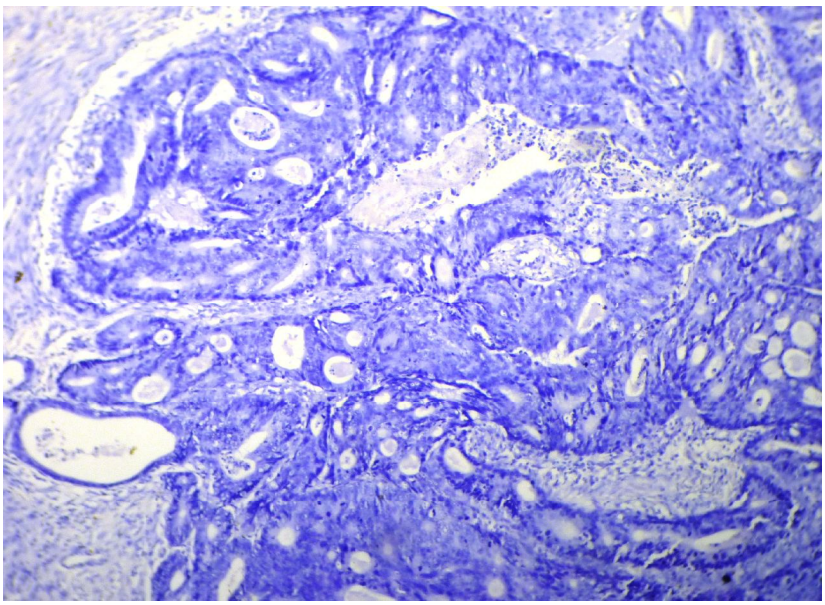


Figure : 9 Moderately differentiated adenocarcinoma – p53 Score 0

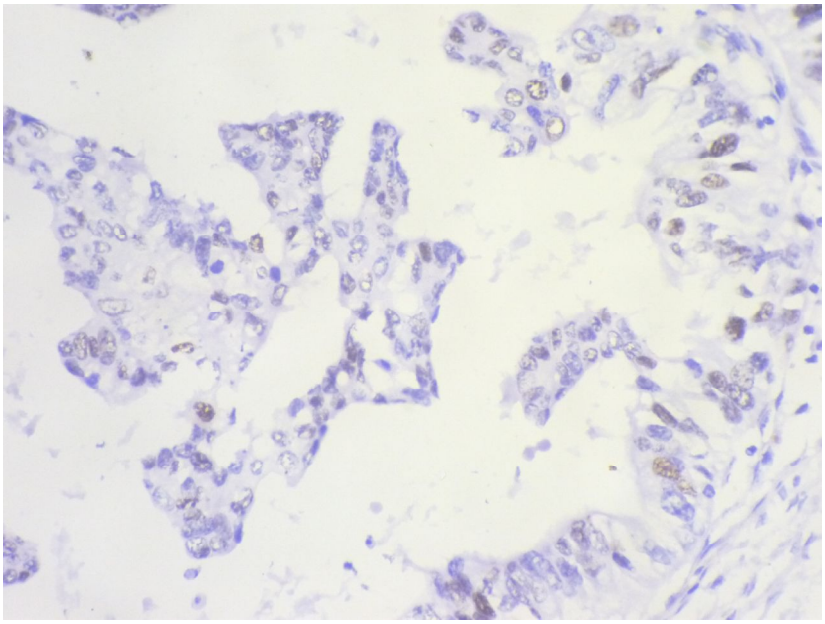


Figure : 10 Moderately differentiated adenocarcinoma – p53 Score 1

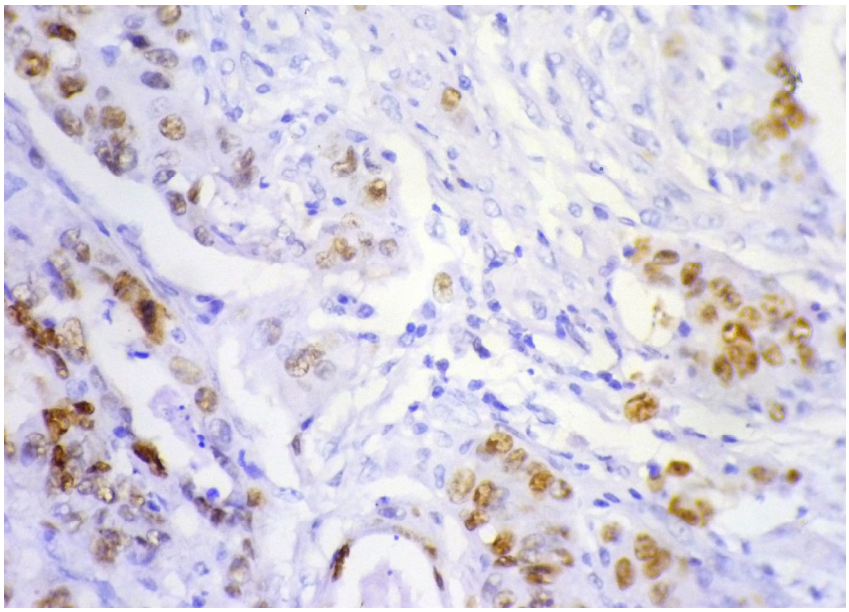


Figure : 11 Moderately differentiated adenocarcinoma – p53 Score 2

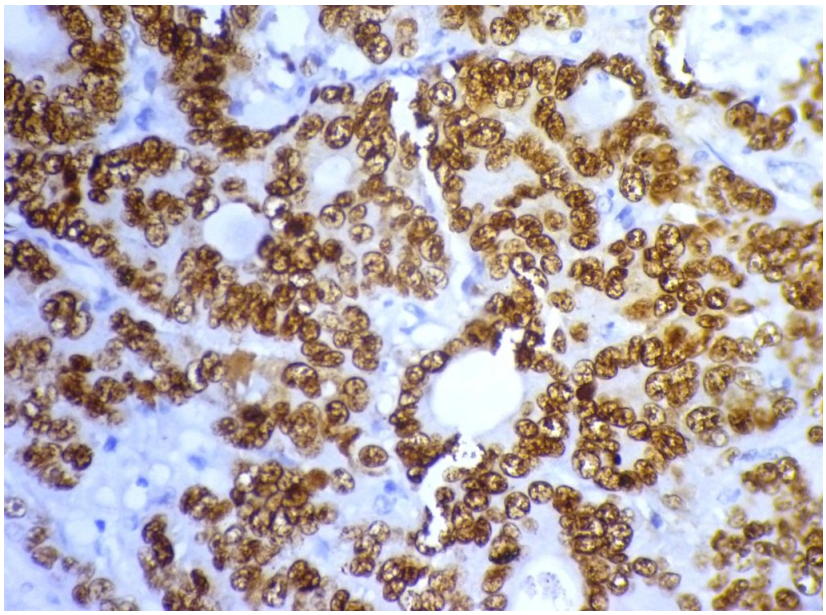


Figure : 12 Moderately differentiated adenocarcinoma – p53 Score 3

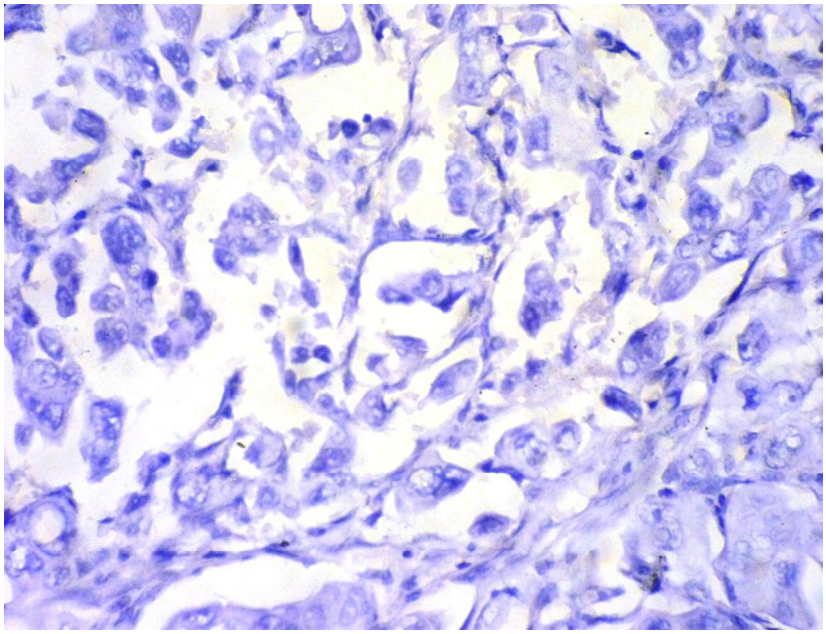


Figure : 13 Poorly differentiated adenocarcinoma – p53 Score 0

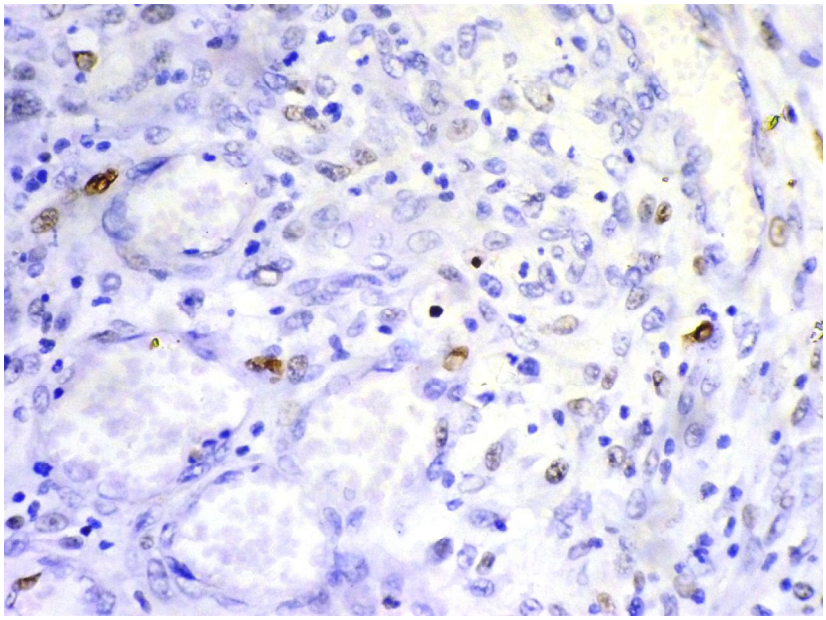


Figure : 14 Poorly differentiated adenocarcinoma – p53 Score 1

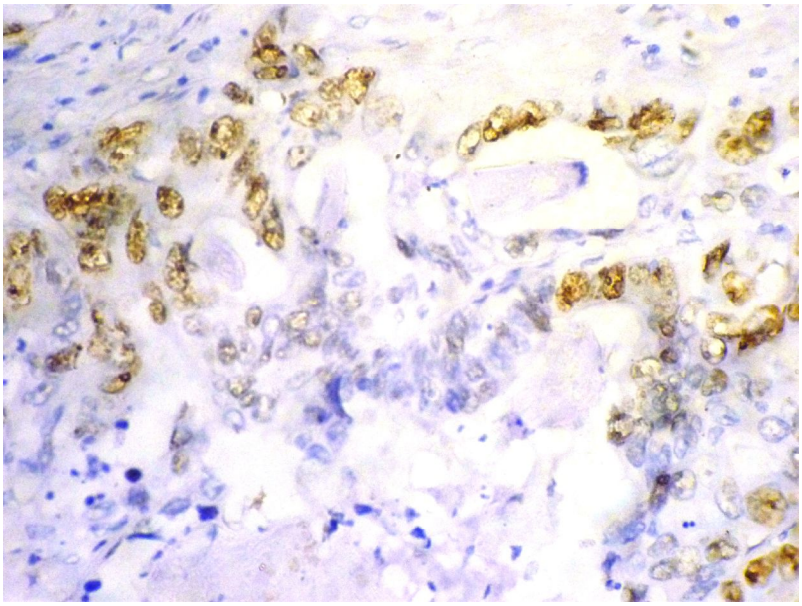


Figure : 15 Poorly differentiated adenocarcinoma – p53 Score 2

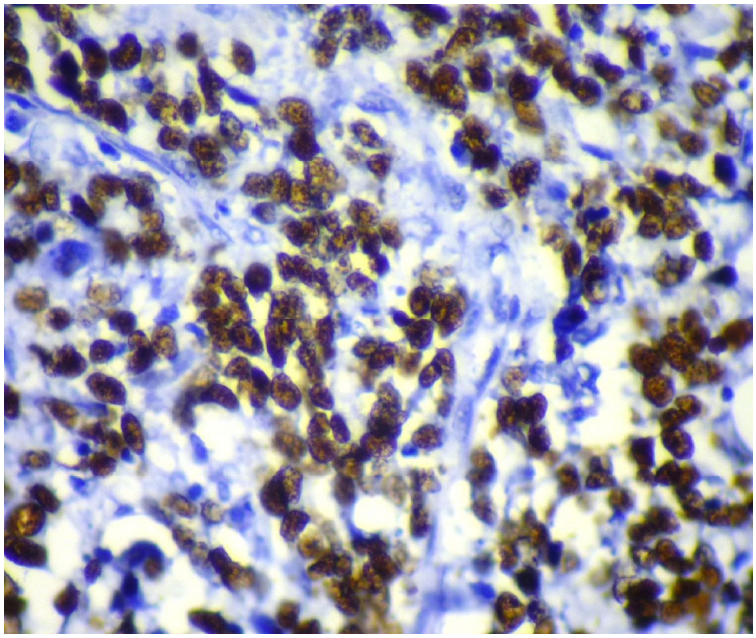


Figure : 16 Poorly differentiated adenocarcinoma – p53 Score 3

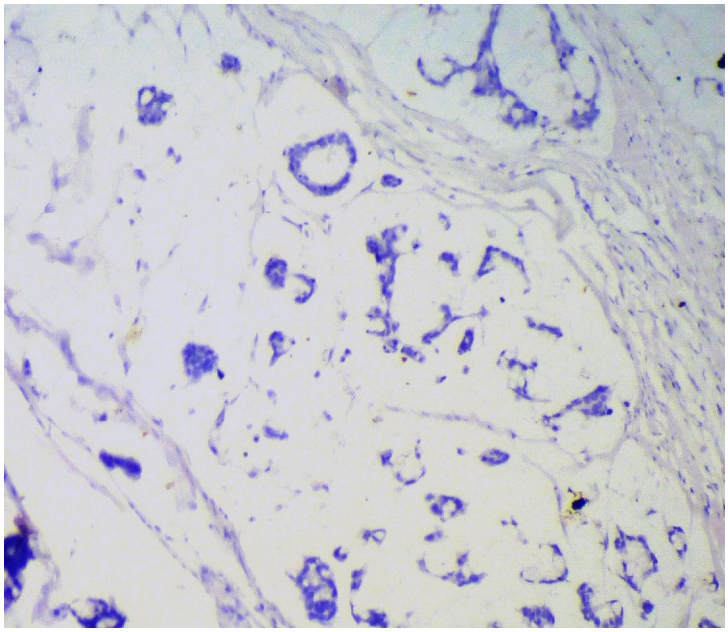


Figure : 17 Mucinous adenocarcinoma – p53 Score 0

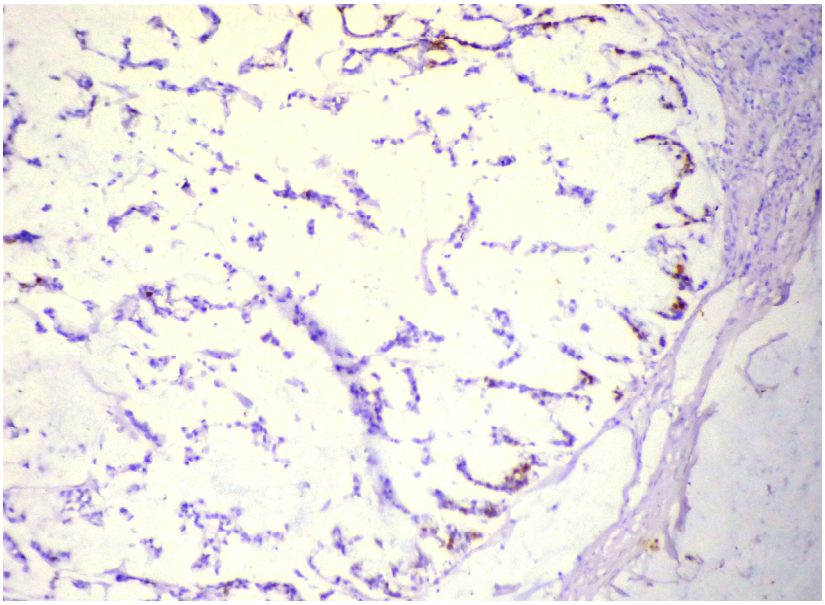


Figure : 18 Mucinous adenocarcinoma – p53 Score 1

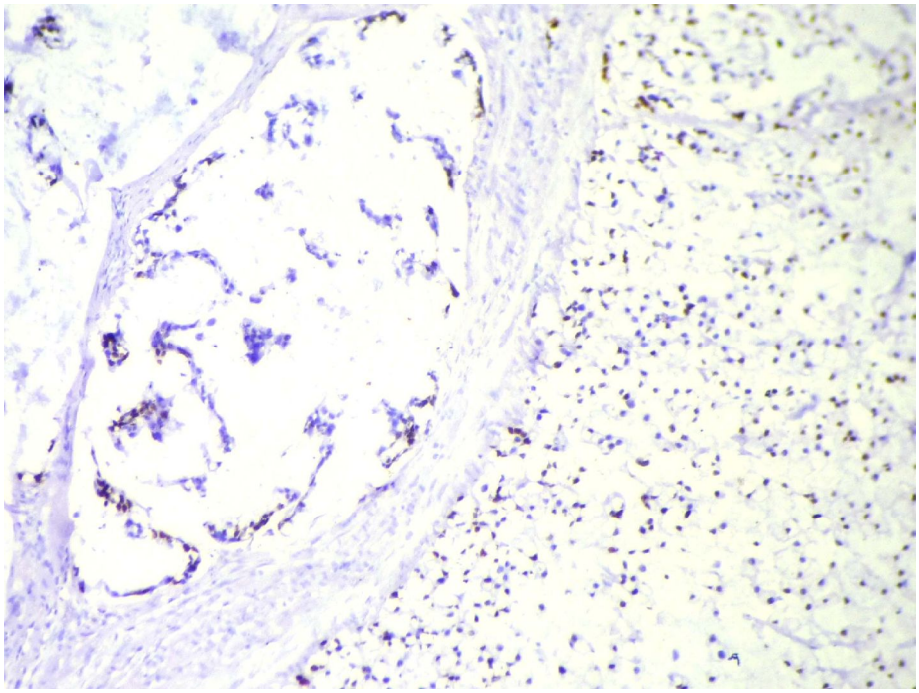


Figure : 19 Mucinous adenocarcinoma – p53 Score 3

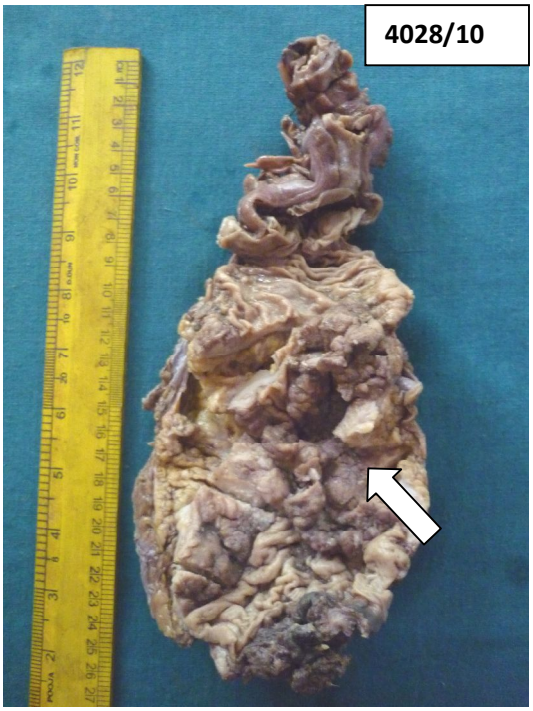


Figure : 20 Abdomino perineal resection – Ulceroproliferative growth in the rectum

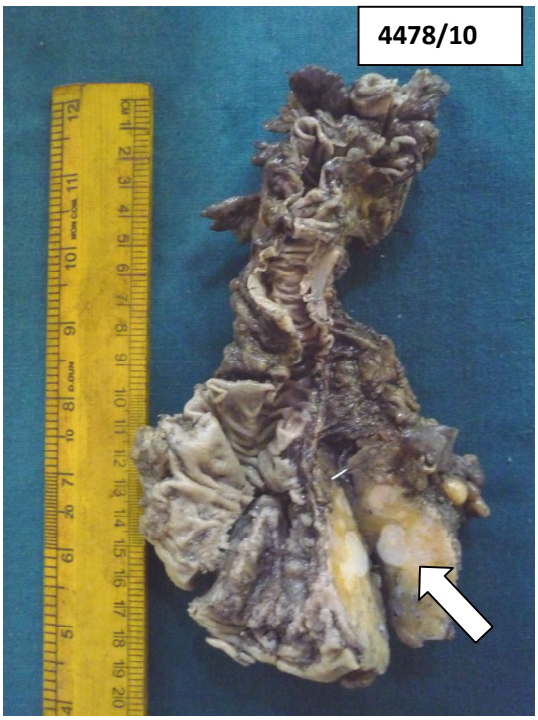


Figure : 21 Low anterior resection – Mucinous tumour in the rectum

DISCUSSION

DISCUSSION

Colorectal carcinoma is by far the most common and most curable cancer of the gastrointestinal tract. More than ninety percent of the cancers of the colorectal region are adenocarcinomas. Males are affected more often than females. The incidence peaks at 60 to 70 years of age and fewer than 20% cases occur before 50 years of age[1]. In this study the age group for colorectal carcinoma was ranging from 24 to 78 years and peak incidence was at 40 to 70 years with a male preponderance.

Rectal carcinomas are more common in Asians[1]. In this study rectum was the commonest site for colorectal adenocarcinoma in all the age groups. Right sided lesions are more likely to present at an older age than distal lesions[1,22]. This is likely due to the fact that right sided carcinomas have a greater chance of being asymptomatic and left sided lesions on the other hand have a greater chance of presenting with bleeding per rectum and changes in bowel habits[1,20] which were the commonest clinical presentation in this study also (52% and 39% respectively). Thus these patients are more apt to recognize a change in their health and seek medical care earlier. These facts are the presumed reasons for presentation of right sided lesions at a later age and advancing stage thus having worse prognosis[17]. In accordance with this fact the present study revealed high frequency of occurrence of higher stage tumours in those above 60 years of age (52.6%) and right sided adenocarcinomas had high stage commonly(53%). 37.5% of cases with right sided adenocarcinoma were above 60 years of age

Common colonoscopic findings in colorectal carcinoma are ulceroproliferative or polypoidal lesions[1] and in this study also similar results were obtained with the ulceroproliferative morphology being the commonest (78%).

Tumours in proximal colons show ulceroproliferative configuration and distal lesions are annular that produce napkin ring like constrictions and luminal narrowing[1,18]. In this study all those tumours which presented as luminal narrowing were present in the rectum (n = 8).

Studies by Soong R et al [50] and Yuan-Tzu Lan et al[54] have shown that tumour size is of no prognostic significance. In this study 67% of the larger tumours (>10 cm) were situated in the caecum which may be due to more space available and late presentation of these tumours.

In general the degree of gland formation is widely regarded as the most important feature in grading. The colorectal carcinoma has been graded as well differentiated (grade I), moderately differentiated (grade II) and poorly differentiated (grade III). Grade IV is somewhat redundant as a category that shows no histological evidence of differentiation and is classified as a separate histological type by the WHO[4].

Despite the lack of standardization and documented interobserver variation in the assessment, the histological grade has been shown repeatedly by multivariate analysis to be a stage-independent prognostic factor and it does not have any correlation with age or sex[47,50]. In this study also there was no significant correlation of age and sex with histological grade of the tumour.

Colorectal mucinous carcinoma is one of the subsets of colorectal adenocarcinoma which is defined according to the WHO as an adenocarcinoma in which a substantial amount of mucin (>50% of the tumour) is retained within the tumour[4]. The 5 year survival rate is 43.1% when compared to non mucinous type (79.4%) in stage II and stage III[58]. In our study most of the mucinous carcinomas were in low stage but the sample size was too low to get a significant correlation. 71% of the mucinous carcinomas were located in the right colon.

In colorectal carcinogenesis two distinct pathways are involved. APC / beta-catenin pathway is associated with classical adenoma-carcinoma sequence and p53 mutation occurs at late stages of tumour progression. Second pathway is microsatellite instability pathway in which the tumours often have mucinous differentiation and are frequently located in the right colon. Hence the mucinous carcinomas are reported to have a lower frequency of p53 mutation.

In this study 120 colectomy specimens received in the Department of Pathology, Stanley medical college during the year 2009 July to 2011 August were examined. 115 cases were adenocarcinomas and 100 cases which had adequate clinical and investigatory data were included in the study. Among these cases 7 cases were of mucinous subtype.

In 50 randomly selected cases p53 expression was studied by immunohistochemistry in various stages and grades of the tumour which included 6 mucinous carcinomas. The expression of p53 was scored as 0 to 3 according to the percentage of cells showing nuclear positivity irrespective of the staining intensity. Results were tabulated and analyzed. For the purpose of statistical analysis stages I and II

were categorized as low stage and stages III and IV were categorized as high stage. Similarly grade III and mucinous carcinomas were classified as high grade and grades I and II were classified as low grade.

This study has shown p53 positivity in 78% of colorectal adenocarcinomas. According to Robbins and cotran[1], 70-80% of colorectal adenocarcinomas show p53 mutation. Yamaguchi et al[47] found the immunoreactivity for p53 in 61% of colorectal carcinomas. George E. Theodoropoulos et al[58] reported nuclear positivity for p53 in 63.4% of colorectal adenocarcinomas. These differences may be due to the use of different scoring systems and interobserver variability.

J Walker et al[23] in his study concluded that the stage is the most accurate prognostic factor for survival and recurrence. In this study there was progressive increase in the p53 score as the stage increases. This association was proved to be statistically significant using Chi-Square test (P value = 0.027). This is in accordance with the study by Flamini ET al[53] and Heide ET al[56].

Also in a recent study by George E. Theodoropoulos[58] it has been found that p53 is overexpressed in 63.4% of colorectal cancer patient population and is significantly associated with advanced stage (P = 0.004).

The present study revealed a lower rate of p53 positivity in mucinous tumours with most of the tumours being negative for p53. Among the positive cases most had a score of 1 but significant correlation could not be obtained due to smaller sample size. This is in concordance with the study by C.Hanski et al [59] who found that only 36% of

mucinous carcinomas have shown p53 positivity by immunohistochemistry but 76% of the non-mucinous adenocarcinomas showed p53 positivity suggesting that mucinous tumours develop by a different pathway. Satoshi Ikeda et al [60] also has observed similar findings in their study.

This study showed no significant association for increasing grade of the tumour with the p53 overexpression which correlates well with the results of the studies by Yamaguchi A et al [47], Soong R et al [50], Yuan-Tzu Lan et al [54] and C.Hanski et al [59]. But high scores were found commonly in well to moderately differentiated tumours (70.6%) than poorly differentiated tumours (29.4%). This is in concordance with the study by Yuan-Tzu Lan et al[54] who found p53 overexpression in 60% of well to moderately differentiated versus 40% of poorly differentiated tumours. This may be due to the fact that p53 overexpression may be reduced as the cells become less differentiated. So p53 can serve as a differentiation marker in colorectal adenocarcinomas.

P53 nuclear positivity in this study was found commonly in left sided tumours (56.4%) with the rectum predominating among them (46.15%). Similar results were found in the study by Antonio Russo et al [45] who found p53 expression in 45% of distal tumours as against 34% of proximal tumours. Also Yuan-Tzu Lan et al [54] in his study has observed a statistically significant correlation between the p53 overexpression by immunohistochemistry and location of the tumor in the rectum.

Since p53 expression has shown a significant association with the stage of the disease and since the stage is a proven prognostic marker in colorectal adenocarcinomas

p53 expression can be used as a prognostic marker to assess the invasiveness and also the metastatic potential of these tumours.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

A total of 120 colectomy specimens were received in the Department of Pathology, Stanley medical college during the year 2009 July to 2011 August. 100 cases of adenocarcinomas diagnosed by hematoxylin and eosin staining of the tissue sections which had adequate clinical and investigatory data were included in the study. Among these cases 7 were of mucinous subtype.

The age group for colorectal adenocarcinoma was ranging from 24 to 78 years with the peak age group of 40 to 70 years. Males were commonly affected. The most common presenting symptoms were bleeding per rectum and altered bowel habits. Colonoscopic finding in most of the tumours was ulceroproliferative growth pattern and the most common site was rectum. All the cases presenting with narrowing of lumen were seen in the rectum.

The tumours were graded as well differentiated, moderate differentiated and poorly differentiated and staged according to the TNM system. High frequencies of tumours in high stage were seen in above 60 years of age group. Higher stage was more common in males and in left sided lesion. Right sided adenocarcinomas had high stage commonly. High grade tumours were more common in males and in those above 60 years of age. Mucinous carcinomas showed slight female preponderance and most of the mucinous carcinomas were located in the right colon.

P53 expression was studied by immunohistochemistry in various stages and grades of the tumour in 50 randomly selected cases which included 6 mucinous carcinomas. Scores were given for p53 expression ranging from 0 to 3 according to the

percentage of cells showing nuclear positivity irrespective of the staining intensity. 78% of the tumours showed p53 positivity. Statistically significant correlation was obtained for p53 overexpression and advanced stage but not for grade or site of the tumour. Poorly differentiated tumours had low expression of p53. Mucinous tumors had lower expression of p53 but the sample size was too low to get a definitive correlation.

To conclude p53 overexpression plays an important role in the progression of colorectal cancer since it correlates strongly with the stage of the tumour and might therefore represents an useful marker of prognosis.

MASTER CHART

SI.NO	HPE NO	AGE	SEX	BPR	ALT BH	LOW	SITE	SIZE	CONFIGURATION	HISTOLOGY	GRADE	STAGE	P53 SCORE
1	2905/09	71	M	Y	N	N	RECTUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	III	
2	2972/09	34	M	N	Y	N	TRANSVERSE COLON	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	I	
3	3008/09	24	M	Y	N	N	RECTUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	II	
4	3014/09	38	M	Y	N	Y	SIGMOID COLON	UPTO 5 CM	POLYP	ADENOCA	I	I	
5	3044/09	72	M	N	Y	N	CAECUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	III	III	3
6	3164/09	74	M	Y	N	N	DESCENDING COLON	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	III	III	2
7	3204/09	68	M	N	Y	N	ASCENDING COLON	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	II	I	
8	3241/09	27	F	Y	N	Y	CAECUM	>10 CM	ULCEROPROLIFERATIVE	MUCINOUS CA		III	0
9	3384/09	48	F	Y	N	N	RECTUM	UPTO 5 CM	NARROWING OF LUMEN	ADENOCA	II	II	
10	3470/09	33	F	Y	N	N	ASCENDING COLON	UPTO 5 CM	POLYP	ADENOCA	I	I	1
11	3508/09	34	M	N	Y	Y	SIGMOID COLON	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	I	I	
12	3680/09	37	F	N	N	Y	RECTUM	UPTO 5 CM	NARROWING OF LUMEN	MUCINOUS CA		I	
13	3841/09	41	F	Y	N	N	SIGMOID COLON	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	II	
14	3900/09	66	M	Y	N	N	TRANSVERSE COLON	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	I	0
15	4086/09	55	M	Y	N	N	RECTUM	UPTO 5 CM	NARROWING OF LUMEN	ADENOCA	II	II	
16	4123/09	39	M	N	Y	Y	RECTUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	I	
17	4255/09	61	M	N	Y	N	RECTUM	UPTO 5 CM	POLYP	ADENOCA	III	III	2
18	4422/09	64	F	Y	N	N	CAECUM	>10 CM	ULCEROPROLIFERATIVE	ADENOCA	I	IV	2
19	4464/09	66	M	Y	N	N	RECTUM	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	I	III	2
20	4500/09	32	M	N	Y	N	ASCENDING COLON	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	I	1
21	4501/09	65	M	Y	N	N	RECTUM	UPTO 5 CM	POLYP	ADENOCA	I	I	0
22	4615/09	67	F	N	Y	N	TRANSVERSE COLON	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	II	IV	2
23	4616/09	55	F	Y	N	N	RECTUM	UPTO 5 CM	NARROWING OF LUMEN	ADENOCA	I	I	0
24	4661/09	35	M	N	Y	N	ASCENDING COLON	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	I	I	0
25	4702/09	66	M	Y	N	N	RECTUM	UPTO 5 CM	NARROWING OF LUMEN	ADENOCA	II	III	
26	4828/09	43	M	N	Y	N	RECTUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	II	I	0
27	4879/09	59	M	Y	N	N	DESCENDING COLON	UPTO 5 CM	POLYP	ADENOCA	II	IV	3
28	5127/09	51	M	N	Y	N	CAECUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	III	III	1
29	5312/09	49	F	Y	N	N	CAECUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	II	IV	
30	5420/09	41	M	Y	N	N	RECTUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	IV	3
31	5553/09	43	M	N	Y	N	RECTUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	IV	3
32	5762/09	55	F	N	N	Y	CAECUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	II	IV	
33	5880/09	54	M	N	Y	N	TRANSVERSE COLON	UPTO 5 CM	POLYP	ADENOCA	I	III	2
34	5960/09	68	F	Y	N	N	SIGMOID COLON	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	II	II	2
35	6004/09	64	M	N	Y	N	RECTUM	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	I	I	3
36	6024/09	67	F	N	Y	N	CAECUM	6-10 CM	ULCEROPROLIFERATIVE	MUCINOUS CA		II	0
37	6079/09	57	F	N	Y	N	RECTUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	I	1
38	6091/09	48	M	Y	N	N	ASCENDING COLON	>10 CM	POLYP	ADENOCA	I	I	1
39	6129/09	45	M	Y	N	N	RECTUM	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	I	IV	3
40	58/10	69	F	N	N	Y	ASCENDING COLON	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	II	II	
41	301/10	56	M	N	Y	N	RECTUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	II	I	

SI.NO	HPE NO	AGE	SEX	BPR	ALT BH	LOW	SITE	SIZE	CONFIGURATION	HISTOLOGY	GRADE	STAGE	P53 SCORE
42	512/10	46	M	N	Y	N	SIGMOID COLON	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	III	
43	758/10	66	F	Y	Y	N	RECTUM	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	II	II	2
44	1018/10	58	M	N	N	Y	CAECUM	>10 CM	POLYP	ADENOCA	III	I	
45	1213/10	76	F	N	Y	N	RECTUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	II	
46	1362/10	68	F	Y	N	N	SIGMOID COLON	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	III	III	
47	1672/10	49	M	N	Y	N	RECTUM	UPTO 5 CM	NARROWING OF LUMEN	ADENOCA	I	I	
48	1831/10	59	F	Y	N	N	DESCENDING COLON	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	II	II	
49	1972/10	41	M	Y	N	N	RECTUM	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	I	III	
50	2212/10	78	F	Y	N	N	TRANSVERSE COLON	UPTO 5 CM	POLYP	ADENOCA	I	I	
51	2416/10	55	F	N	N	Y	RECTUM	UPTO 5 CM	NARROWING OF LUMEN	ADENOCA	II	II	
52	2607/10	33	M	N	Y	N	ASCENDING COLON	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	III	III	
53	2712/10	39	M	N	Y	N	RECTUM	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	II	II	1
54	2735/10	46	M	Y	N	N	SIGMOID COLON	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	II	0
55	2819/10	34	M	Y	N	N	RECTUM	UPTO 5 CM	POLYP	ADENOCA	III	II	
56	2961/10	46	M	Y	N	N	ASCENDING COLON	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	I	
57	3052/10	37	F	N	Y	N	RECTUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	IV	3
58	3106/10	48	M	N	Y	N	CAECUM	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	II	IV	
59	3266/10	45	M	N	Y	N	TRANSVERSE COLON	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	II	II	3
60	3276/10	44	F	Y	N	N	ASCENDING COLON	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	I	IV	2
61	3314/10	48	M	N	Y	N	RECTUM	6-10 CM	POLYP	ADENOCA	II	II	
62	3416/10	72	F	Y	N	N	RECTUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	I	
63	3482/10	56	M	N	Y	N	SIGMOID COLON	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	III	II	
64	3609/10	44	M	Y	N	N	RECTUM	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	II	IV	
65	3732/10	71	F	N	N	Y	RECTUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	IV	3
66	3800/10	45	M	N	Y	N	ASCENDING COLON	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	II	IV	
67	3844/10	44	M	Y	N	N	CAECUM	>10 CM	ULCEROPROLIFERATIVE	MUCINOUS CA		II	1
68	3879/10	58	M	N	Y	N	RECTUM	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	III	II	2
69	3901/10	56	M	Y	N	N	RECTUM	UPTO 5 CM	POLYP	ADENOCA	II	II	
70	4009/10	47	M	N	Y	N	SIGMOID COLON	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	I	3
71	4028/10	59	M	N	Y	N	RECTUM	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	II	II	3
72	4183/10	56	M	Y	N	N	RECTUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	I	2
73	4217/10	49	F	N	Y	N	DESCENDING COLON	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	II	III	
74	4308/10	43	M	Y	N	N	RECTUM	6-10 CM	NARROWING OF LUMEN	ADENOCA	I	I	
75	4337/10	38	M	N	Y	N	CAECUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	III	3
76	4478/10	42	M	Y	N	Y	RECTUM	UPTO 5 CM	ULCEROPROLIFERATIVE	MUCINOUS CA		I	0
77	4510/10	36	M	N	Y	N	RECTUM	6-10 CM	POLYP	ADENOCA	I	IV	2
78	4691/10	65	M	Y	N	N	CAECUM	6-10 CM	ULCEROPROLIFERATIVE	MUCINOUS CA		II	3
79	4701/10	69	M	Y	N	N	RECTUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	II	II	
80	4780/10	44	F	Y	N	N	SIGMOID COLON	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	I	
81	4889/10	57	M	N	Y	N	ASCENDING COLON	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	I	IV	2
82	4938/10	66	F	Y	N	N	RECTUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	II	III	0

Sl.NO	HPE NO	AGE	SEX	BPR	ALT BH	LOW	SITE	SIZE	CONFIGURATION	HISTOLOGY	GRADE	STAGE	P53 SCORE
83	5096/10	69	M	Y	N	N	RECTUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	III	IV	
84	5128/10	62	F	N	Y	N	SIGMOID COLON	6-10 CM	POLYP	ADENOCA	II	I	
85	5231/10	63	F	N	Y	N	CAECUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	II	I	
86	5378/10	75	F	Y	N	N	RECTUM	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	III	II	
87	5524/10	64	M	N	Y	N	SIGMOID COLON	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	II	IV	
88	5702/10	46	F	Y	N	N	CAECUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	III	I	
89	5790/10	64	M	N	N	Y	ASCENDING COLON	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	II	IV	
90	5804/10	61	F	Y	N	N	SIGMOID COLON	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	III	I	
91	5955/10	68	M	Y	N	N	CAECUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	I	IV	2
92	6010/10	58	F	N	N	Y	ASCENDING COLON	6-10 CM	ULCEROPROLIFERATIVE	MUCINOUS CA		III	1
93	6115/10	69	M	Y	N	N	RECTUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	III	IV	3
94	105/11	72	M	Y	N	N	CAECUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	III	IV	3
95	199/11	55	F	Y	N	N	RECTUM	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	II	III	3
96	512/11	75	M	N	N	Y	CAECUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	III	IV	
97	630/11	76	M	Y	N	N	ASCENDING COLON	>10 CM	ULCEROPROLIFERATIVE	ADENOCA	III	II	0
98	741/11	71	M	Y	N	N	SIGMOID COLON	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	III	IV	
99	839/11	73	M	Y	N	N	RECTUM	UPTO 5 CM	ULCEROPROLIFERATIVE	ADENOCA	III	IV	3
100	1278/11	42	F	N	N	Y	CAECUM	6-10 CM	ULCEROPROLIFERATIVE	ADENOCA	II	I	

HPE – Histopathological examination

BPR – Bleeding per rectum

ALT BH – Altered bowel habits

LOW – Loss of weight

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INSTITUTIONAL ETHICAL COMMITTEE, STANLEY MEDICAL COLLEGE, CHENNAI-3

Title of the Work : Immunohistochemical evaluation of colorectal malignancies - A study of 100 cases.

Principal Investigator : Dr. V.Sindu
Designation : M.D. Pathology Post Graduate
Department : Department of Pathology
Government Stanley Medical College,
Chennai-1

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 04.02.2011 at the Modernized Seminar Hall, Stanley Medical College, Chennai-1 at 2PM

The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
2. You should not deviate form the area of the work for which you applied for ethical clearance.
3. You should inform the IEC immediately, in case of any adverse events or serious adverse reaction.
4. You should abide to the rules and regulation of the institution(s).
5. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of the work.

23/2/14

MEMBER SECRETARY,
IEC, SMC, CHENNAI

23/2/2011

IMMUNOHISTOCHEMICAL EVALUATION OF COLORECTAL MALIGNANCIES

A STUDY OF 100 CASES

ABSTRACT

Colorectal adenocarcinoma is a major cause of mortality and morbidity worldwide. Adenocarcinoma accounts for over 90% of the malignant tumours of the colorectal region. Adenoma-carcinoma sequence theory is accepted in carcinogenesis of colorectal carcinoma. Mutation of p53 occurs at the time of transition from adenoma to carcinoma. In this study 100 cases of adenocarcinomas of the colorectal region were analysed for varied clinical presentations, colonoscopic findings, site and size distribution. In 50 randomly selected cases with various histological grades and TNM stages p53 expression was studied by immunohistochemistry which included 6 mucinous carcinomas. Scores were given for p53 expression which were ranging from 0 to 3 according to the percentage of cells showing positivity irrespective of the staining intensity. Statistically significant correlation was obtained for p53 overexpression and advanced stage but not for histological grade and site of the tumour. Mucinous tumours had a lower expression of p53. Since p53 correlates strongly with the stage of the tumour it may represent a useful prognostic marker.

KEY WORDS : Colorectal adenocarcinoma, p53, mucinous carcinoma.